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STUDIES ON THE RELATIONSHIP OF GROWTH TEMPERATURE TO DNA SYNTHESIS IN THE SALIVARY GLANDS OF *DROSOPHILA MELANOGASTER*

by

© JOHN BLAIN BELL

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The undersigned certify that they have read, and recommend to The Faculty of Graduate Studies for acceptance a thesis entitled "Studies on The Relationship of Growth Temperature to DNA Synthesis in The Salivary Glands of Drosophila melanogaster" submitted by John Blain Bell in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Culturing Drosophila melanogaster at low culture temperatures produces larger than normal larvae which contain larger salivary glands. The cell number of these glands is fixed early in embryonic life (Sonnenblick, 1950) and consequent growth of the glands is by growth in cell size alone. The above facts suggest that it is reasonable to expect that the individual nuclei from glands grown at lower temperatures might contain more DNA.

This idea has been tested by growing cultures of larvae over a range of temperatures and subjecting samples of glands from each temperature to autoradiography and photometry. The autoradiography, using H^3-thymidine, was done to determine the fraction of salivary gland nuclei that were synthesizing DNA. The photometry, after the method of Patau (1952), was done to determine the relative amounts of DNA found in the nuclei of each sample. White prepupae were used because they represent the stage where the highest level of polyteny is found. They also represent the shortest easily identifiable portion in the developmental cycle of Drosophila melanogaster which makes it easier to obtain a homogeneous sample.

Lowering the culture temperature did not result in an increase in the fraction of nuclei of individual glands found to be synthesizing DNA, as measured autoradiographically, nor did it result in an increase of the ultimate amount of DNA synthesized, as measured photometrically. The autoradiographic studies did, however, indicate that there is great variation in the fraction of cells of individual glands that are synthesizing DNA. It is suggested that this could be inherited. Variation was not as evident in the photometric data, but it was found that there is very

significant between gland variation in DNA content of the nuclei of the lowest temperature sample. It is suggested that this could represent a partial escape from a canalized control where latent genetic differences have become manifest due to an environmental stress.

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INTRODUCTION AND LITERATURE REVIEW

The important role of deoxyribose nucleic acid (DNA) in inheritance and cellular metabolism is now widely accepted. It follows from this that the amount of DNA found in cells must be under some sort of control. In general, it has been found that the amount of DNA per cell is constant, within limits, from tissue to tissue within an organism and also that the amount of DNA per cell is a species characteristic (Swift, 1949; Mirsky and Ris, 1951). The basis for quantitative estimation of DNA content of cells is the 'C' value (concentration of DNA per haploid chromosome complement of an organism). According to Pollister et al (1951) there is in most cells a constant relation between the DNA content and the number of chromosome sets it contains. For example, Swift (1950a) studied several non-dividing somatic tissues, germinal tissue and dividing tissue in the mouse. With respect to DNA content, he found one class of nuclei common to all of the somatic tissues. Mature sperm were found to have one-half the DNA content of the common somatic class. In addition he found nuclei in somatic tissue with twice and four times the DNA content of the common class. The common somatic class was designated as the diploid (2C); sperm as haploid (C); and the others as tetraploid (4C) and octaploid (8C) respectively. In the dividing tissue nuclei were found that ranged between 2C and 4C in DNA content. This is a common feature of mitotically active cells; the DNA replicates in interphase to double the 2C level and it is not until subsequent separation of the two daughter cells that the 2C level is restored. In non-dividing tissue, cells with over

2C amounts of DNA are found which have been interpreted as having extra chromosomal complements and can be considered as triploid, tetraploid et cetera.

In dividing tissue, cells with the 4C amount of DNA commonly divide to become two cells; therefore it can be said that cell division usually follows DNA replication. DNA replication not followed by cell division results in the production of cells with greater amounts of DNA than the diploid value. Such multiplication of chromonemata or chromosomes without division of the nucleus is defined as endomitosis (Geitler, 1941; in Swanson, 1957). Polyteny is a special case of endomitosis in which the DNA replicates, often repeatedly, but the chromonemata remain fused to each other to give a multi-stranded chromosome (DeRobertis, 1965).

Polytene chromosomes are widespread in the larval tissues of Diptera. They are known to occur in the nuclei of fat bodies, malpighian tubules, intestinal tracts, trachea, brain ganglia, muscles and salivary glands. It has been suggested that nuclei of all cells fated to be histolyzed during metamorphosis are likely to contain polytene chromosomes (Cooper, 1938). The most general statement that can be made about polytene chromosomes is that they usually occur in end cells.

Polytene nuclei have been considered to be in permanent prophase (DeRobertis, 1965). However, since prophase cells show little or no DNA synthesis and yet it is well demonstrated that polytene chromosomes are very active in synthesizing DNA (Plaut, 1963; Gabrusewycz-Garcia, 1964; Pavan, 1965) they might, more logically be considered as in interphase.

There are three notable features of polytene chromosomes apart from their great size. Homologous chromosomes tend to show somatic pairing, often amounting to complete fusion. They also display a distinctive pattern of bands that is so regular in its appearance and sequence that detailed cytological maps have been constructed (Bridges, 1935). The presence of DNA in these bands has been demonstrated by the feulgen reaction (Heitz and Bauer, 1933; in Swift, 1962). The interbands were also found to be slightly feulgen positive. This finding has recently been confirmed by autoradiography (Plaut and Nash, 1964) and by fluorescence microscopy (Wolstenholme, 1965). The third structural feature of polytene chromosomes is the presence of puffs. Puffing occurs in specific regions of chromosomes and can be correlated with specific tissues and stages of development. It is now widely accepted that the puffs represent genes in an active metabolic state (Beermann, 1965). There is evidence that in some Diptera puffing is also related to localized synthesis of a limited amount of additional DNA (for references see Swift, 1962; Rasch, 1967), but to date there is no evidence for this in Drosophila (Swift, 1962).

The synthesis of DNA in polytene chromosomes of Drosophila has been studied autoradiographically. Two kinds of asynchrony of replication have been noted. Not all cells within any one salivary gland were found to be replicating DNA at one time; in cells synthesizing DNA, it is frequently found that discrete regions of label may be interspersed between regions without label (Plaut, 1963; Gay, 1963; Rudkin and Woods, 1959; Plaut, Nash and Fanning, 1966). The last mentioned authors have also determined an ordered sequence of replication of these regions for

part of the genome. In addition to the discontinuously labelled chromosomes, nuclei containing only continuously labelled chromosomes are observed. There is some agreement that this stage of continuous labelling represents a stage early in the period of DNA synthesis in polytene chromosomes (Plaut, Nash and Fanning, 1966; Berendes, 1966; Nash and Bell, 1968; Rodman, 1968).

Tissues which contain polytene nuclei typically grow by increase in cell size rather than cell number. In Drosophila salivary glands, cell number is determined by the time the gland primordium is formed in the embryo and no mitosis occurs thereafter. Growth of these glands to their relatively large size in the early prepupae is due exclusively to increase in cell size (Sonnenblick, 1950). The minimum range of variability in number of cells per salivary gland in Drosophila melanogaster wild-type is 107 to 144 (Hadorn and Faulhaber, 1962).

The level of polyteny achieved before histolysis sets in varies from tissue to tissue in Drosophila. Among the organs suitable for experimentation, it is in the salivary glands that it reaches its highest level (Swift and Rasch, in Alfert, 1954). One of the earliest attempts to estimate the degree of polyteny in Drosophila salivary glands was by Hertwig (1935) who arrived at figures of 256C and 512C for the largest nuclei. The degree of polyteny was thought to be of the order of one to two thousand on the basis of the relative volumes of salivary gland nuclei and diploid nuclei (Painter, 1941). An early cytophotometric study by Kurnick and Herskowitz (1952) placed the level of DNA at approximately one thousand times the diploid value. Since the advent of the two wavelength technique of cytophotometry (Patau, 1952) there have been other

studies on polytene level in salivary glands. At least two workers place the highest level of polyteny in wild-type Drosophila as that resulting from the eighth replication (Rudkin, 1965; Rodman, 1967). Also using wild-type Drosophila others have found nuclei of 1024C, corresponding to the ninth replication. (Welch, 1957; Swift, 1962.)

There is evidence that the highest polytenic level found in wild-type Drosophila salivary glands can be surpassed in certain circumstances. Using non-genetic means Hadorn et al. (1964) have demonstrated that by transplanting third instar larval salivary glands into the abdomens of adult females some nuclei are obtained that contain up to 3.5 times the average found in controls. This was demonstrated photometrically after a twenty day culturing of the implanted glands. Rodman (1967b) has demonstrated photometrically that nuclei corresponding to higher polytenic levels than she found in wild-type are found in the mutant strain tu-h (tumorous-head). At standard temperature this strain has an extended larval period due to a failure or delay in pupation. Indeed, Church and Robertson (1966) have shown that even in Drosophila wild-type there is genetic polymorphism with respect to amount of DNA synthesized, albeit by the total organism rather than just the larval salivary glands.

The ultimate amount of DNA synthesized in the salivary glands may even be partially under environmental control. Since insects can be classified energetically as being examples of an open system (Clark, 1967) it is probable that their internal control mechanisms are subject to modifications from environmental factors such as culture temperature. It has been known for years that growing Drosophila at lower temperatures produces larger larvae. Since the number of cells in the salivary gland

is established very early in embryonic life, subsequent growth is the result of increasing cell size. Therefore, it is reasonable to expect that the largest larvae should have the largest cells and consequently the largest chromosomes and more DNA. Indeed, there is good reason to believe that by growing Drosophila larvae at low temperatures one might be able to induce a higher level of polyteny in the salivary glands, since it is known that chromosomes from larvae grown at low temperatures are superior for cytological examination because of their larger size (Schultz, 1936; Bridges, 1938).

However, visual observation of the size of chromosomes is not an accurate means of determining amount of DNA. The feulgen reaction coupled with cytophotometry offers an accurate means of testing the possibility that larvae grown at lower temperatures might possess cells which attain higher levels of polyteny. By comparing the relative DNA values of samples of nuclei taken from prepupae reared at a range of different temperatures it should be possible to ascertain whether there is more DNA in nuclei from lower temperature samples. The results of such a study are presented below.

The speculation that lowered culture temperatures might result in higher levels of polyteny in salivary gland nuclei is not incompatible with consequent development to imagos. Since function of larval salivary glands ceases upon histolysis of the glands during pupal metamorphosis an extra level of polyteny could quite logically be expected not to affect the adult phenotype. Moreover, from present experimental evidence that the attained level of polyteny in nine hour prepupae varies from the fifth to the eighth replication among the nuclei of one salivary gland

(Rodman, 1967a), there is at least a suggestion that the level of polyteny in these nuclei is not critical; for, at that time, histolysis of the gland is well advanced (Bodenstein, 1950) and therefore, not all of the nuclei can reach the highest level in any case.

Rodman (1967b) relates the capacity for initiation of new cycles of DNA synthesis in the salivary gland nuclei to duration of the larval state. She believes that new initiations can take place as long as the larval state exists. It can easily be demonstrated that changing the culture temperature of developing Drosophila can modify the total time taken for growth. Growing the cultures at lowered temperatures increases the time of development, often quite dramatically (see results). If extending the duration of larval life in itself is sufficient to produce a higher level of polyteny, then growing the larvae at lower temperatures should produce this effect. Rodman (1967c) was unable to demonstrate higher levels of polyteny in salivary glands of prepupae grown at a lower temperature. Her results should not be considered conclusive, however, since she used a very small sample. Comparing larvae grown at 17.5°C. and 25°C. she finds no extra level of polyteny in the nuclei from the lower temperature. However a great many more nuclei among the low temperature sample reach the highest level of polyteny. This trend in itself justified the continuation of the study presented here.

It is known that the percentage of cells synthesizing DNA drops continuously throughout late larval life (Nash and Bell, 1968; Rodman, 1968) and continues to drop rapidly in prepupal life (Plaut, Nash and Fanning, 1966; Rodman, 1968). It has also been shown that the percentage of continuously labelled chromosomes drops with increased larval age. If

the speculations with regard to continuously labelled chromosomes being closest to the initiatory phase of DNA synthesis are correct then the commencement of new rounds of replications diminishes in late third instar larvae and essentially ceases in early prepupal life. If lowering the culture temperature does indeed allow extra cycles of replication to take place, then it might be expected that under some circumstances this would result in alterations of the numbers of cells synthesizing DNA in early prepupal life. The fraction of nuclei synthesizing DNA in early prepupal life was autoradiographically determined to examine this possibility and, also, because it would seem to be a biological correlate which might prove exceedingly useful in interpretation of photometric measurements of DNA concentration.

MATERIALS AND METHODS

A. Culture Media

A standard corn-meal medium was used for routine stock cultures. Approximately 25 ml. of the medium was used in half-pint bottles. 4" x 1" specimen vials containing approximately 5 ml. of a yeast-agar-chloramphenicol medium (Nash and Bell, 1968) were used for experimental cultures. Egg-laying dishes were made by pouring a molten agar solution into small petri dishes. The composition of these media is shown below.

Cornmeal medium:

Sodium Potassium tartrate	24 gm.
Calcium chloride	1.5 gm.
Agar	18.5 gm.
Dried Brewer's Yeast	88 gm.
Cornmeal	210 gm.
Dextrose	173 gm.
Sucrose	87 gm.
Distilled water	3500 ml.
Propionic acid	35 ml.

Yeast-agar-chloramphenicol medium: (Nash and Bell, 1968)

Brewer's Yeast	100 gm.
Sucrose	100 gm.
Agar	10 gm.
Propionic acid	10 ml.
Chloramphenicol	100 mg.
Distilled water	1000 ml.

Egg-laying medium:

Agar 1.5% w/v H_2O

Propionic acid 1% v/v H_2O

B. Fly Culture

Drosophila melanogaster (Oregon wild-type) flies were used throughout this study. Stocks were maintained by routine mass transfer. All of the breeding stock cultures were incubated at 20°C. in half-pint bottles containing the standard corn-meal medium.

Larvae used for preparations of salivary gland nuclei were obtained from these stocks in the following manner. Flies, usually at least five days old, were selected for egg-laying and pre-fed for at least twelve hours in normal culture bottles supplemented with a paste of brewer's yeast. After this time the flies were transferred to empty culture bottles, over which egg-laying dishes were taped. After two to three hours of laying, flies were removed from the dishes and dead flies or other detritus were cleared from the surface, leaving only the eggs. The eggs were placed in 20°C. incubators and hatched in approximately thirty-six hours.

Fifty newly hatched larvae were placed in culture vials. These larvae were reared at appropriate temperatures in Millipore embedding ovens operating inside a larger incubator set at a low temperature. Cultures of larvae were raised at seven temperatures ranging from 12.5°C. to 27.5°C.

A preliminary study was done to establish the mean time to pupation at each of these temperatures in order to ascertain the most favorable time to obtain maximum numbers of white prepupae.

C. Selection and use of white prepupae for experiments

With the exception of one control experiment in which third instar larvae were used, young, that is, white prepupae were used throughout this study. This is because it is the prepupal stage where the highest level of polyteny is achieved, as histolysis of the salivary glands begins soon after puparium formation. White prepupae offer the additional advantage of being the shortest easily identifiable portion in the developmental cycle of the fly from time of hatching to eclosion. At 25°C. the time spent as a white prepupa represents about .5% (1/192 hours) of the total time to eclosion (Bodenstein, 1950). Being able to choose the experimental sample from this very short but specific stage in the life history adds a desirable control to the autoradiographic studies since it is known that there is a steady drop in DNA synthetic activity over the few hours between late third instar larvae and prepupae (Rodman, 1968).

The white prepupae were also selected as to their sex because there is evidence that males and females contain different amounts of DNA (Nigon and Daillie, 1958; Church and Robertson, 1966b). Also Berendes (1966) has shown evidence for a difference in the replication patterns of DNA in the two sexes by autoradiographic means. Males were selected over females because they are easier to identify. This is because their relatively large gonads are clearly visible through the transparent prepupal case, whereas the female gonads are small and are mostly covered by fat bodies.

D. Preparation of microscope slides

All slides used in this study were coated with .5% gelatin solution in order to achieve better adherence of the tissue. They were

allowed to dry in an upright position and stored in wooden slide boxes until required for use.

E. Preparation of Autoradiographs

The salivary glands of the male white prepupae were excised in a drop of insect Ringer (Ephrussi and Beadle, 1936) on a clean slide. After excision, the glands were transferred to a 5 ml. plastic petri dish containing 3 ml. Ringer's solution to which 10 μc/ml. of 5-Methyl-H^3-thymidine (New England Nuclear Corporation, NET-027X Lot #261-165-2, specific activity 16,600 mc/mmole) had been added and incubated from twenty to twenty-seven minutes. These incubations were carried out at room temperatures (22°C.) or, exceptionally, at 12.5°C.

The glands were fixed and squashed in 45% acetic acid. Special care was taken to squash the tissue very lightly so that the chromosomes would remain clumped together, facilitating later determinations of presence or absence of label over individual nuclei. 10% neutral formalin proved to be inadequate for fixation, since subsequent staining with aceto-orcein produced undifferentiated preparations.

The tissue was then frozen by dipping the slides in liquid nitrogen and, after immediate removal of coverslips, the slides were immersed in a bath of 3:1 ethanol-acetic acid for post-fixation. The slides were passed through an alcohol series to water, into 45% acetic acid, stained for five minutes in aceto-orcein (2% orcein in 60% acetic acid), washed in 45% acetic acid, dehydrated through the alcohol series and mounted in a drop of Euparal (Gurr) for storage. Coverslips were removed from permanent slides by soaking them in absolute alcohol over-

night at room temperature. Before autoradiography slides were taken down to water through an alcohol series. Often slides were autoradiographed immediately after staining, in which case they were washed thoroughly in distilled water and dehydration, mounting, etc. were omitted.

Slides were covered with Kodak AR-10 stripping emulsion and exposed for the length of time required by each experiment. A preliminary experiment was done using glands from 17.5°C. prepupae to determine a satisfactory exposure time required to reach a maximum level of nuclei displaying significant labelling. See Figure 1 (Page 14) for these preliminary results. It was found that a plateau of maximum labelling was reached after approximately a sixteen day exposure. To ensure that all slides were likely to be on this plateau, the minimum exposure time of twenty-six days was selected for half of the slides while the other half were given 50% more exposure time (39 days). This sampling technique provides a check that the maximal level of nuclear labelling has been ascertained. After the required exposure, the slides were developed with Kodak D19b and fixed in Kodak acid fix. To illustrate that this procedure gives good incorporation with relatively low background see Figure 2 (Page 15).

The developed slides were analyzed by counting the number of labelled nuclei out of the total number of nuclei. Classifying very lightly labelled nuclei as labelled was quite arbitrary, as the density of label deemed sufficient to be considered nonbackground varied from slide to slide and therefore the criterion for considering a nucleus to be labelled or not consisted of comparing it to the background for that

Figure 1. Variation in the number of cells judged to exhibit tritium labelling after autoradiographic exposures of different duration. Autoradiographic conditions were as described in the text. Each point represents cells from between 12 and 20 glands taken from male white prepupae grown at 17.5° C. The size of these samples ranges from 914 - 1651 cells.

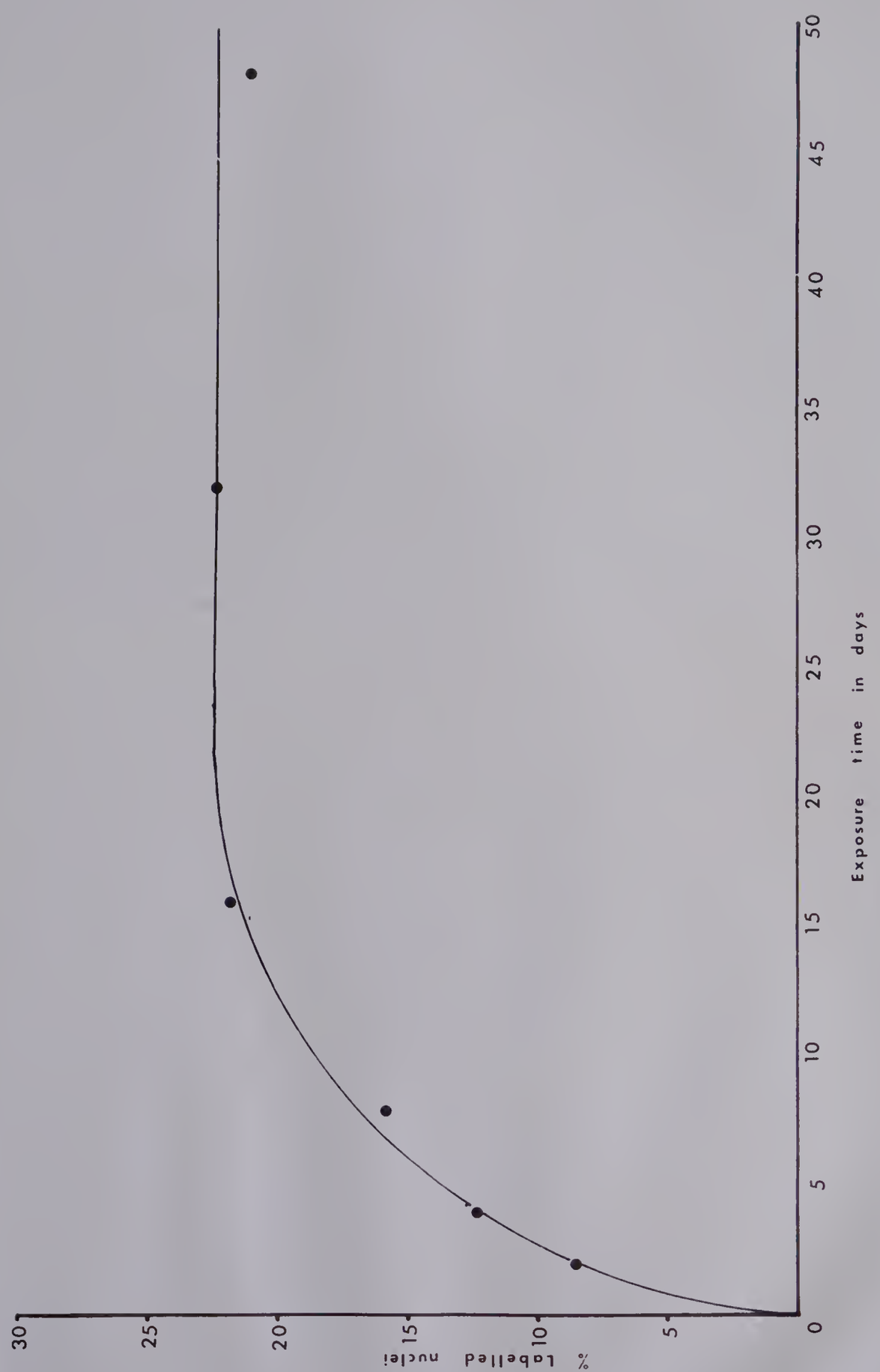
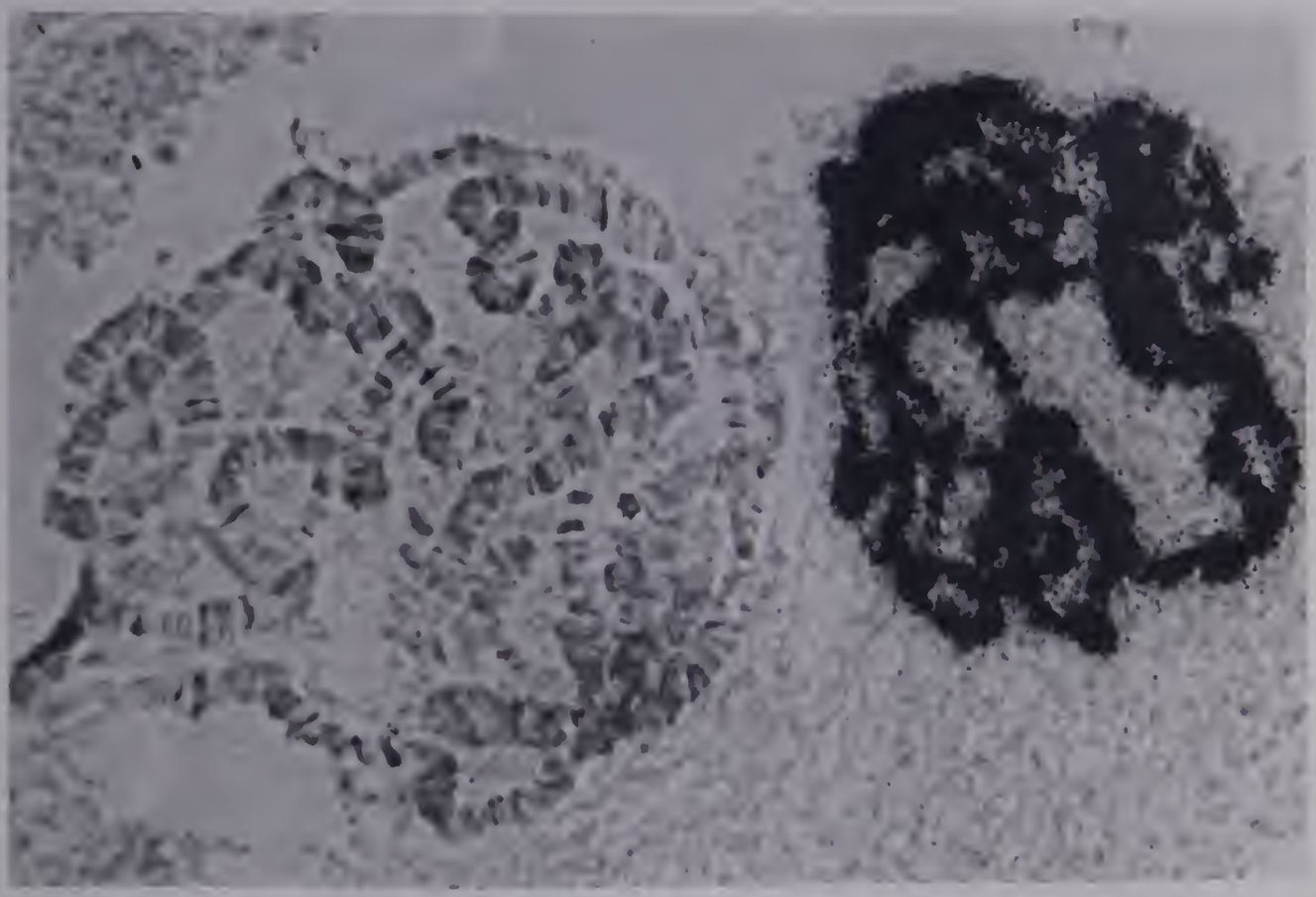

% labelled nuclei
30
25
20
15
10
5
0
5
10
15
20
25
30
35
40
45
50
Exposure time in days

Figure 2. Above. Two salivary gland nuclei, from the same gland, exhibiting different autoradiographic behavior. The one nucleus is unlabelled except for background grains, while the other shows an extremely dense concentration of grains.

Below. A nucleus which exhibits light labelling but which is certainly above the background level. All three nuclei were subjected to the same autoradiographic procedure, as described in the text. Exposure time was 26 days. Magnification x1500.



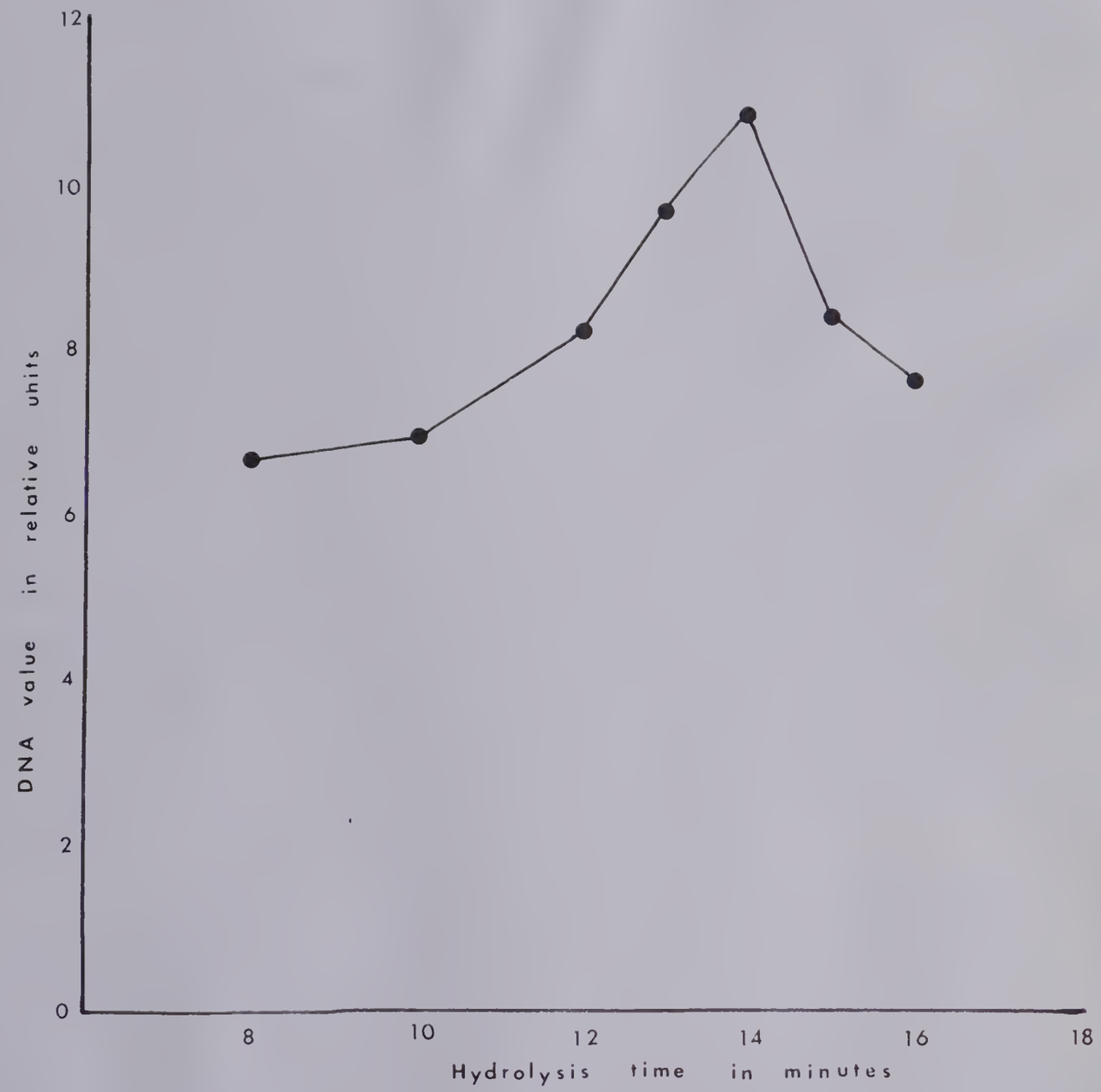
particular slide. See Figure 2 for an illustration of heavy and light label over nuclei. 5-Methyl-H^3-thymidine is known to be incorporated specifically into DNA with no detectable shunting to RNA (Rudkin and Woods, 1959). That autoradiographic labelling represents labelling of DNA can be illustrated by its removal after deoxyribonuclease treatment but not after ribonuclease or protease treatment (Plaut, 1963).

F. Preparation of slides for photometry

The cytology involved for preparing feulgen stained slides was the same except for the omission of incubation in H^3-thymidine and the substitution of the 45% acetic acid fixative with 10% neutral formalin. This fixative has the advantage of reducing spreading of the tissue when squashing is carried out, which makes it a desirable fixative for photometric work where compact areas are easier to measure. Cytoplasmic staining is not a significant factor as it was in the autoradiography since the feulgen technique, if used properly, is highly specific for DNA.

The feulgen technique used here was devised by DeTomasi (1936). Staining solutions were made according to his specifications using Fisher certified basic fuchsin (total dye content 99%). However, the staining schedule of DeTomasi was not followed rigorously. Hydrolysis was carried out using 1N HCl in a controlled 60°C. waterbath for fourteen minutes. A preliminary experiment varying hydrolysis times showed 14 minutes to result in maximal staining (See Figure 3, Page 17). This hydrolysis time differs from the four to five minutes suggested by DeTomasi. Another departure was rinsing the slides in 60°C. distilled water before hydrolysis, rather than cold 1N HCl as suggested by DeTomasi. This serves a

Figure 3. Relationship of stainibility with the feulgen technique to hydrolysis time. Male white prepupae salivary gland nuclei were prepared and measured photometrically as described in text. Hydrolysis was carried out in 1N HCl at 60° C. Fifty cells were measured in each sample.


12
10
8
6
4
2
0
DNA value in relative units
8
10
12
14
16
18
Hydrolysis time in minutes

two-fold function. It brings the slides up to 60°C. so that when they are placed in the 60°C. hydrolysis bath they will not cool it off. Secondly, exposure to the acid is limited to the actual time the slides are in the hydrolysis bath.

After a fourteen minute hydrolysis, the slides were stained for four hours in solution 'A' (DeTomasi, 1936; in Conn et al, 1965) which consists of 0.5 gm. of basic fuchsin per 100 ml. water, decolorized by adding 10 ml. N HCl and 0.5 gm. $K_2S_2O_5$ and standing overnight. If solution A was not completely decolorized by this treatment, 0.5 gm. of activated charcoal was added and the solution was filtered. From solution A, the slides were passed quickly to the first of a series of three coplin jars, each containing solution 'B' (DeTomasi, 1936; in Conn et al, 1965). Solution B, prepared just before use, consists of 5 ml. of 10% aqueous $K_2S_2O_5$ and 5 ml. of N HCl per 100 ml. distilled water. The slides were kept ten minutes in each of these jars in succession, keeping the jars closed. After staining and differentiation the slides were washed in tap water for ten minutes, dehydrated through an alcohol series and mounted in Euparal (Refractive index = 1.485).

The photometric measurements were taken using a Zeiss photomicroscope with a microphotometer attachment. The lens used was a Planar 2.5/0.08. After the method of Patau (1952), readings of every nucleus were taken at two wave-lengths. 490 mμ and 514 mμ were the wave-lengths selected for the experiments here since, according to Patau, employing wave-lengths at which absorption of the dye is less than maximal (maximum is at 560 mμ) cuts down on the distributional error and stray-light error inherent in any photometric technique. Each nucleus

was measured three times at each wave-length and the measurements were averaged in the calculations. The nuclei that were measured were selected by taking a rough count of the total number of nuclei on a slide and then selecting a random sample of fifteen from each slide by counting off appropriate numbers of nuclei in a routine scanning procedure. Fifteen such slides were measured at each of the seven temperatures used, making a sample of 225 measured nuclei at each temperature.

Calculations of DNA values from the readings was done by means of Patau's (1952) formula:

$$\text{Amount of dye} = L_1 \cdot \frac{1}{2 - L_2/L_1} \ln_{10} \frac{1}{L_2/L_1 - 1}$$

where L_1 and L_2 are the absorbancies at 490 mμ and 514 mμ respectively. If the field size is varied or if the absolute amount of DNA is to be calculated other parameters must be added to the formula. In this study only one field size was used and no attempt was made to calculate absolute DNA concentrations, since a relative measurement is sufficient to determine if there is more DNA in nuclei from the lower temperatures. A Digital PDP-8/S computer was used to calculate the relative DNA values of all measured nuclei.

It is generally agreed that if the feulgen nucleal reaction is properly carried out it can be used as a quantitative stain for DNA (Swift, 1950a; Ris and Mirsky, 1949). The minimum conditions are satisfied in the experiments described here. These include use of one fixative and a set pH, controlled temperature and set time of hydrolysis. To test the general reliability of the measurements of the white prepupae, a sample of 125 male mid third instar larval nuclei grown at 20°C. were measured. The results were compared with the results of the 20°C. male white prepupae.

RESULTS

Relationship of development rate to culture temperature:

In order to ascertain the degree to which culture temperature can affect development rate a preliminary experiment was carried out. The aim was to calculate the mean time to pupation at each of the culture temperatures, which facilitated the planning of later experiments.

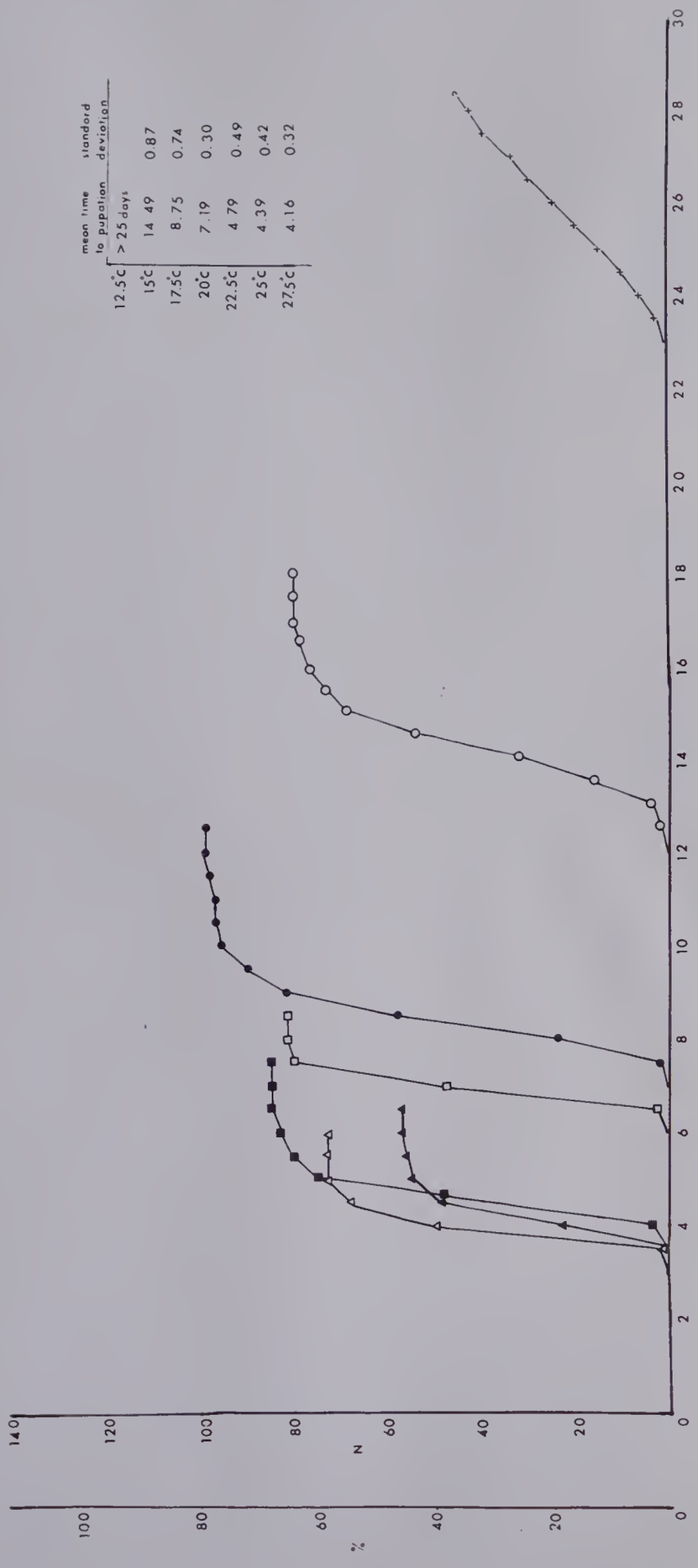
Five culture tubes, each containing 25 newly hatched larvae, were placed at each of the seven experimental temperatures. The tubes were inspected for new puparia at twelve hour intervals. With the exception of the 12.5°C. cultures, the cultures were inspected in this manner until puparium formation stopped. The 12.5°C. cultures were inspected up to 28 days at which time there were still a few large third instar larvae. In a later experiment at 12.5° C. puparium formation extended over seven days. The results of the experiment described above are illustrated in Figure 4 (Page 21). Within the limits of this experiment, a lower development temperature lowers larval development rate. Although it is quite possible that changing the culture temperature affects viability level, this experiment was not meant to point this out. I suggest that the varying levels of viability found in this experiment are due primarily to handling technique and that this precludes any attempt to correlate culture temperature with viability in this situation.

Autoradiography

A sample of salivary glands from male white prepupae grown at each of the culture temperatures was labelled by incubating in H^3-thymidine and slides were prepared as outlined in the Materials and Methods. These

Figure 4. Relationship of culture temperatures to larval development rates. 25 newly hatched larvae were placed in each of five vials at each of seven culture temperatures making a sample of 125 larvae at each temperature. The ordinate has two scales, one expressing development in percentages and the other giving total numbers that have pupated by a given time. Number of days to pupation represents time from placing newly hatched first instar larvae in vials, not the time from egg deposition. The mean time to pupation and the standard deviations are denoted for each temperature in the inset.

Legend:
- 12.5°C. — + + + —
- 15°C. — o o o —
- 17.5°C. — • • • —
- 20°C. — □ □ □ —
- 22.5°C. — ■ ■ ■ —
- 25°C. — ▲ ▲ ▲ —
- 27.5°C. — △ △ △ —


mean time to pupation
standard deviation
12.5°C > 25 days
15°C 14.49 0.87
17.5°C 8.75 0.74
20°C 7.19 0.30
22.5°C 4.79 0.49
25°C 4.39 0.42
27.5°C 4.16 0.32
%
N
Number of days to pupation

slides (40 from each culture temperature) were made into autoradiographs and an analysis was done to determine whether there is any significant variation in the percentage of nuclei labelled from the various samples. From the results of a preliminary experiment (see Figure 1) it was shown that essentially any exposure time over 16 days would give satisfactory estimates of the frequency of labelled nuclei, but as explained in Materials and Methods 26 days and 39 days were chosen. The sample of 40 slides from each temperature was divided into two groups; one group was exposed for 26 days and the other for 39 days.

The results of the analysis of the autoradiographs are outlined in Table I. Due to normal variations in the number of nuclei per gland and also to loss of tissue through experimental procedures, the total number of nuclei counted is different for each of the culture temperatures. Since the percentage of nuclei that were labelled after 26 and 39 days exposure were generally similar it was concluded that both gave satisfactory estimates of the real frequency of labelled nuclei for each of the temperatures and therefore, that it would be proper to combine the data from both exposures (column 7, Table I, Page 23). The frequency of labelling in all of the samples as shown in Table I is considerably higher than the maximum found in the preliminary experiment as portrayed in Figure 1. This is primarily due to an alteration in counting technique. The results of Table I include a great many nuclei of a type that were not counted in the preliminary experiment. These include a class of very small polytene nuclei, from the duct of the glands, as well as fragments of nuclei that were judged to represent at least one-half of the genome. Fragments of the nuclei that were judged to represent less than one-half of the genome were still excluded, as in

Table I - Percentages of labelled* nuclei from male white prepupae grown at various temperatures

Culture temperature	26 day exposure			39 day exposure			Combined data
	No. of cells counted	No. of cells labelled	% labelled cells	No. of cells counted	No. of cells labelled	% labelled cells	% label
12.5°C	1544	479	31.0	1667	483	29.0	30.0
15.0°C	1828	555	30.4	1224	362	29.7	30.0
17.5°C	1881	515	27.4	2210	654	29.6	28.5
20.0°C	1962	660	33.6	2551	860	33.7	33.7
22.5°C	1357	412	30.4	1731	546	31.5	31.0
25.0°C	1841	526	28.6	2228	664	29.8	29.3
27.5°C	2198	596	27.1	2006	544	27.1	27.1
				heterogeneity $\chi^2_{(12)}$ =		56.59	
				p		.005	
12.5°C**	1243	240	19.3	1011***	279	27.6	-

* Incubations were carried out at room temperature (22°C.) in 5-Methyl-H^3-thymidine

** This incubation was carried out at 12.5°C. on prepupae grown at 12.5°C.

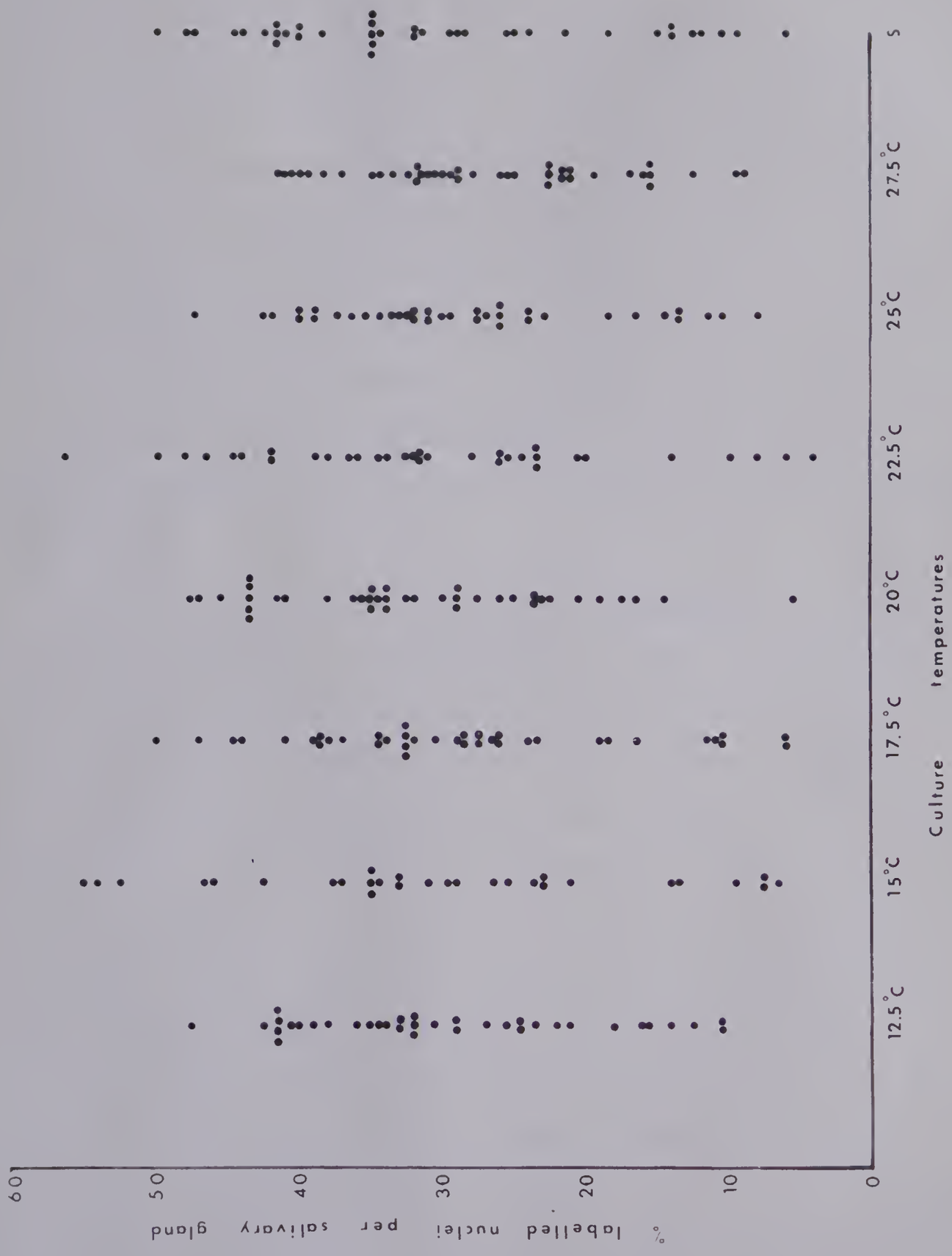
*** Exposure time for this sample was 52 days rather than 39 days.

the preliminary experiment. It is apparent that a proportionately larger fraction of these additional nuclei exhibited labelling.

Table 1 also shows the result of using glands grown at 12.5°C. followed by incubation at 12.5°C. From this it is concluded that low temperature incubation lowers the rate of thymidine incorporation (19.3% for the 26 day exposure as opposed to 31.0% in the comparable 22°C. incubation). However, it does not seem to lower the total number of cells that are incorporating, because a 52 day exposure of glands incubated at 12.5°C. resulted in 27.6% of the cells labelled. By inference, then, the change of temperature that resulted from carrying out the incubations at room temperature (22°C.) did not alter the number of nuclei synthesizing DNA.

From column 7 of Table 1, it can be seen that there is no consistent trend relating percentage of cells labelled to temperature. However, it can be shown that there is significant heterogeneity between temperatures (χ^2 - Table 1). This heterogeneity could be accounted for by a phenomenon observed in this experiment. It was found that salivary glands within a sample at any one temperature could exhibit as much as a tenfold variation in the percentage of labelled cells (5-50%; see Figure 5, Page 25). This finding could explain the significant heterogeneity. It would be easy to bias a sample by fortuitously selecting more of the glands from one extreme of the observed variation. However, the power of this explanation can be minimized by the observation that there is very little difference in the frequency of label between sub-samples at any temperature. If sampling error were the cause of the significant heterogeneity between the samples from the different temperatures then it would also be expected

Figure 5. The percentage of labelled nuclei in individual glands at every culture temperature. Each point represents one salivary gland. The number of nuclei in each gland varies from 27 to 165. The sample "S" represents glands (selected from all temperatures) with over 125 nuclei and demonstrates that the variability found in labelling frequency is not due to tissue loss. The data in Table I was computed from the same sample of cells as this figure.

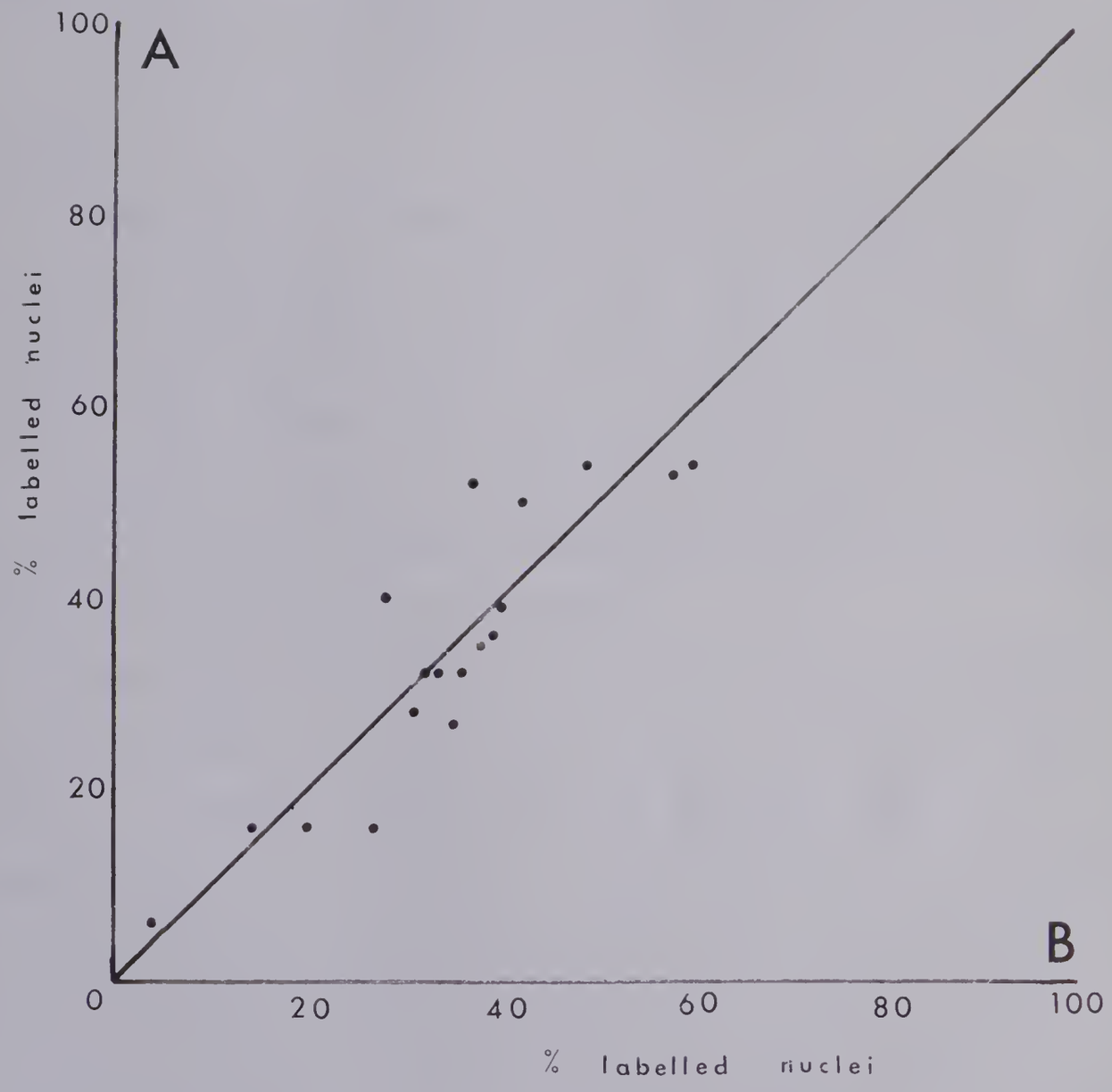

% labelled nuclei per salivary gland
60
50
40
30
20
10
0
12.5°C
15°C
17.5°C
20°C
22.5°C
25°C
27.5°C
S
Culture temperatures

to result in a similar level of heterogeneity between the sub-samples.

The remarkable degree of variation between individual slides in a sample was an unexpected result. It was possible that the source of this variation was merely experimental artefact caused by comparing glands with varying amounts of tissue. If whole glands were not always present on the slides, the missing portions could be heavily labelled or virtually unlabelled and thereby account for the variation found. Column "S" of Figure 5 shows a sample of salivary glands drawn equally from all temperatures but at random from all slides bearing 125 nuclei or over. Assuming that any preparation with over 125 nuclei represents an intact gland, it can be seen from column "S" that even among presumably intact glands the same degree of variation in labelling frequency is exhibited.

Salivary glands are paired structures in Drosophila. By studying the labelling frequency in separated glands of a pair, it is possible to gain further information as to the nature of the observed tenfold variation in label frequency. Pairs of salivary glands were excised, but not separated from each other, and incubated together in H^3-thymidine Ringer solution. After incubation was completed the glands were separated and prepared on separate slides by the usual method (see materials and methods). Figure 6 (Page 27) compares the frequencies of labelling found in members of salivary gland pairs. The frequency of label in gland "A" of a pair is plotted against the frequency of label found in gland "B" of a pair. Since different numbers of nuclei are found on these slides, statistical analysis is difficult. It can be seen, however, that the data clusters about a straight line, passing through the origin with a gradient of 45^{o}, which would be expected if there was a high

Figure 6. Comparison of labelling frequency between the two glands of salivary gland pairs. Each point represents the frequency of gland "A" of a pair compared to the frequency of gland "B" of a pair. No consideration was given to the number of cells found on individual slides. The range was from 40 cells, representing extensive loss of tissue, to 160 cells. The autoradiographic procedure is described in the text.


100
A
80
60
40
20
0
% labelled nuclei
20
40
60
80
100
B
% labelled nuclei

degree of correlation between the two glands in a pair. This suggests a possible biological basis to the degree of variation found in labelling frequency and certainly rules out experimental artefacts that occurred any time after fixation of the glands as possible explanations. This result also excludes death of segments of the glands due to handling as explanations for the observed variation in label frequency and reinforces the conclusion that the variation is not due to tissue loss.

Photometry

A sample of fifteen feulgen-stained slides was prepared from each of the culture temperatures as outlined above. Fifteen nuclei on each slide were measured photometrically as previously described. Figure 7 (Page 29) shows a typical sample of the distribution of cell sizes and staining densities found in the feulgen-stained slides used in this experiment. After the photometric measurements were taken the relative amounts of DNA per nucleus were calculated.

Figure 8 (Pages 30 and 31) shows the results of all the calculations for DNA content, for each of the samples, in the form of histograms. It is apparent that there are no marked differences in the average DNA content of cells grown at lowered temperatures. The differences in the mean DNA values for each culture temperature (see Table II, Page 32) are small and there is no evident trend. However, from the histograms of Figure 8 it does seem that there is some tendency for nuclei in the 12.5^{o}C. sample to reach the highest DNA class as evidenced by a greater frequency of nuclei with 20 and over relative DNA content.

Figure 9 (Pages 33-36) shows the photometric data for every measured nucleus on every slide at each of the temperatures. The distribution of DNA values

Figure 7. A photomicrograph demonstrating the range of nuclear sizes and staining densities that can be found in one salivary gland treated by the feulgen technique described in the text. Magnification x200.

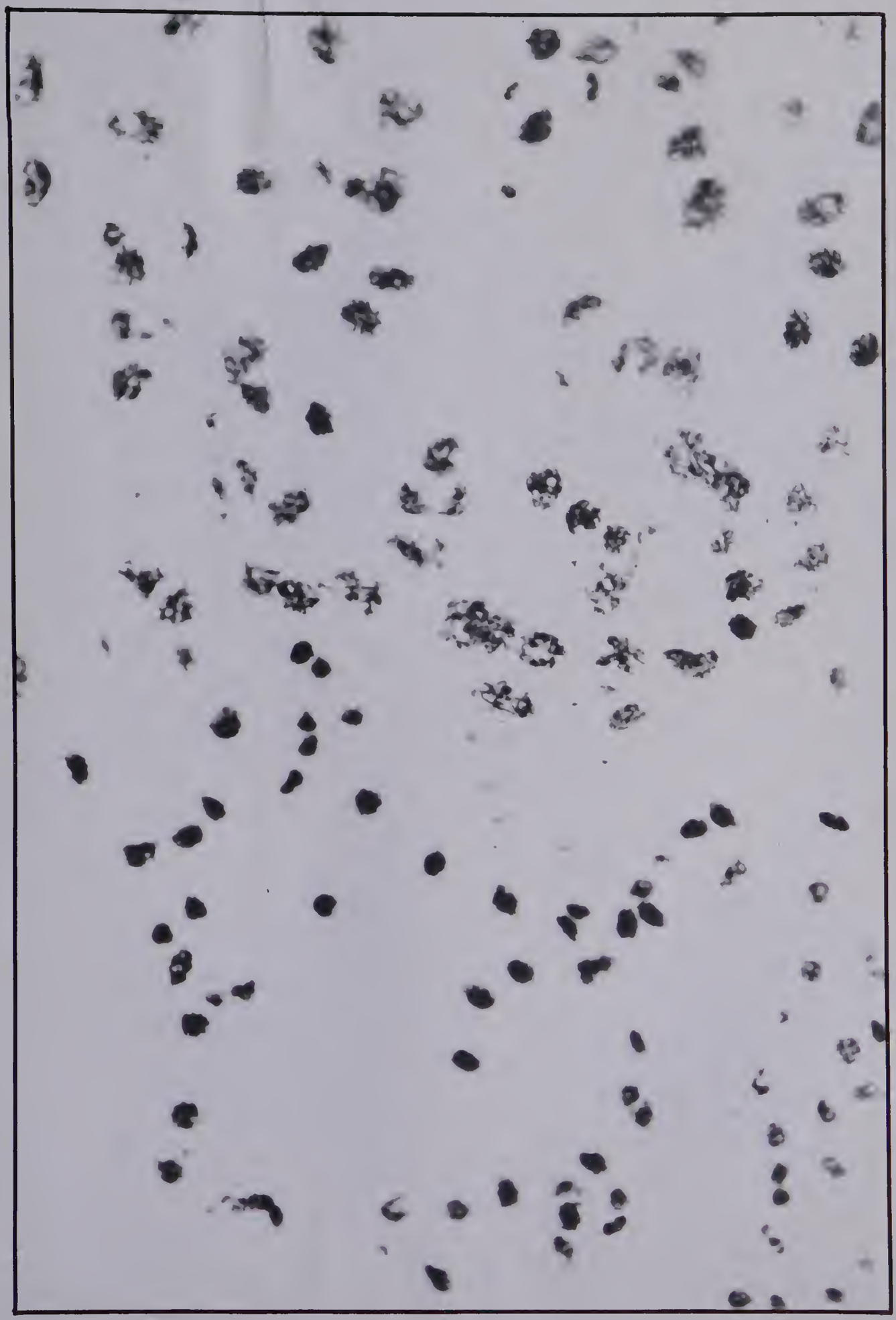
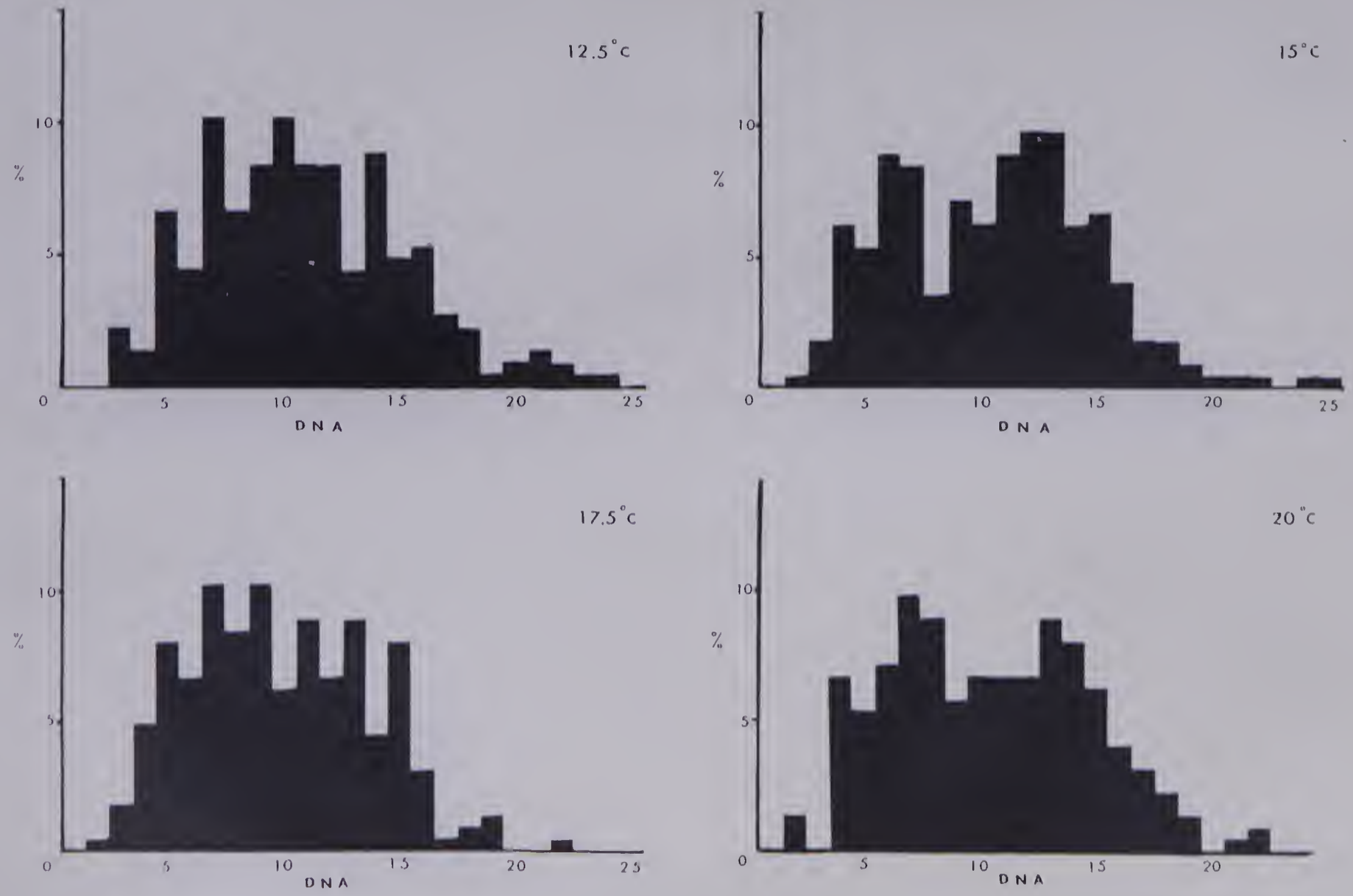


Figure 8. The distribution of nuclei from each of the culture temperatures according to their relative DNA contents. Each sample consists of 225 salivary gland nuclei from male white prepupae selected and measured as described in the text. The vertical axis of the histograms represents the percentage of each total sample that falls within a given range of relative DNA content, expressed in relative units.


12.5°C
15°C
17.5°C
20°C
%
10
5
0
5
10
15
20
25
DNA

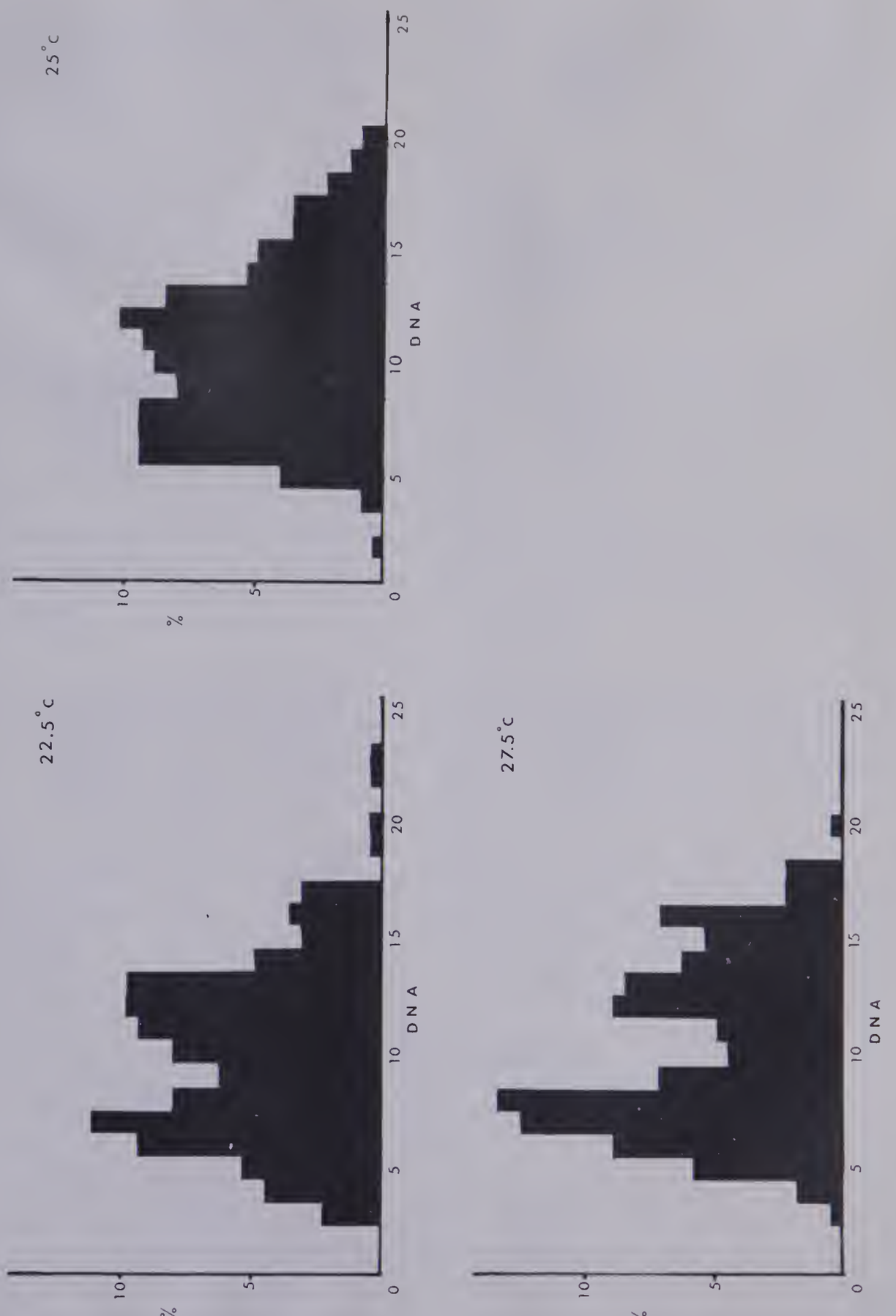

25°C
22.5°C
27.5°C
%
DNA
0
5
10
15
20
25

Table II. Analysis of variance of the results of feulgen photometry

12.5°C. (mean DNA value : 10.91)

Source	Sum of Squares	df	Variance
Total	4074.28	224	--
Between slides	1495.35	14	106.81
Residual*	2578.93	210	12.28

F = 8.69
$p < .01$

15°C. (mean DNA value : 10.42)

Source	Sum of Squares	df	Variance
Total	4177.75	224	--
Between slides	590.41	14	42.17
Residual	3587.34	210	17.08

F = 2.46
$p < .01$

17.5°C. (mean DNA value : 9.84)

Source	Sum of Squares	df	Variance
Total	3360.68	224	--
Between slides	571.40	14	40.81
Residual	2789.28	210	13.28

F = 3.07
$p < .01$

20°C. (mean DNA value : 10.44)

Source	Sum of Squares	df	Variance
Total	4043.31	224	--
Between slides	246.78	14	18.07
Residual	3796.53	210	17.62

F - 1.03
$p > .05$

22.5°C. (mean DNA value : 9.95)

Source	Sum of Squares	df	Variance
Total	3263.93	224	--
Between slides	748.89	14	53.49
Residual	2515.04	210	11.97

F = 4.46
$p < .01$

25°C. (mean DNA value : 10.63)

Source	Sum of Squares	df	Variance
Total	3118.29	224	--
Between slides	307.00	14	21.92
Residual	2811.29	210	13.38

F = 1.63
$p > .05$

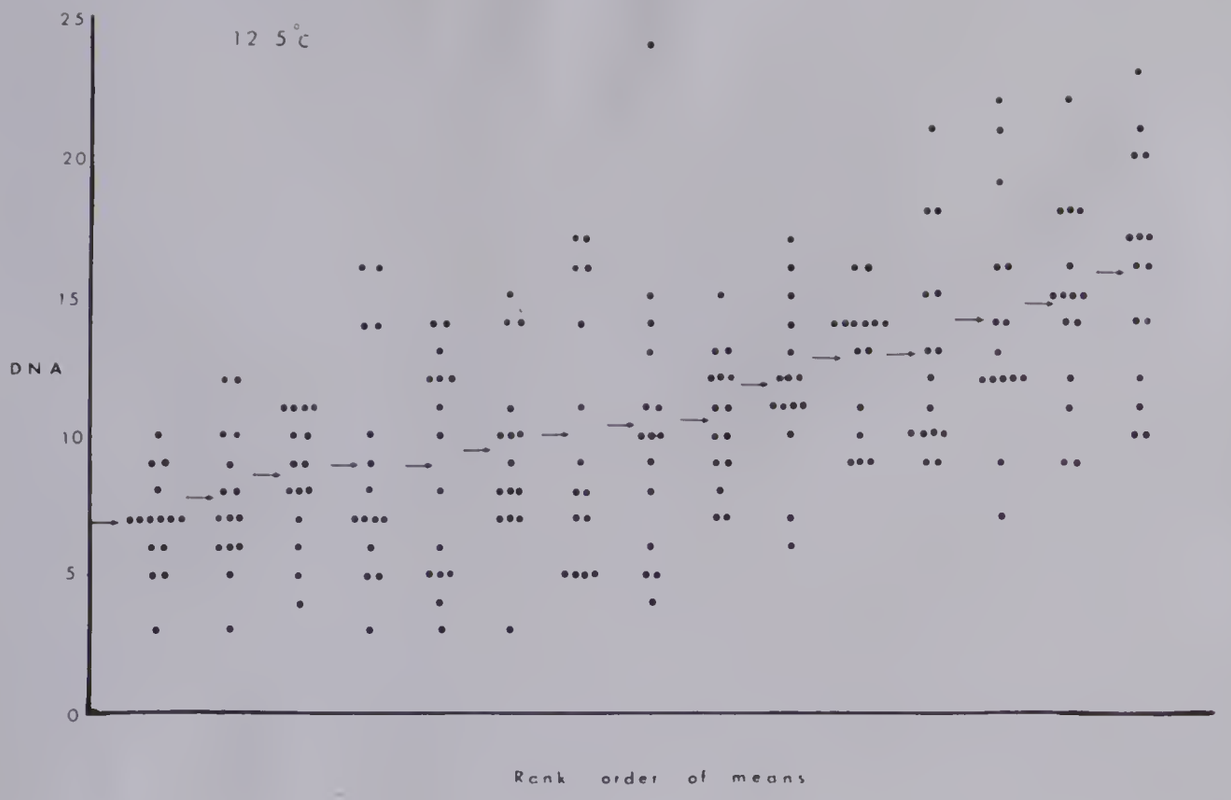
27°C. (mean DNA value : 10.32)

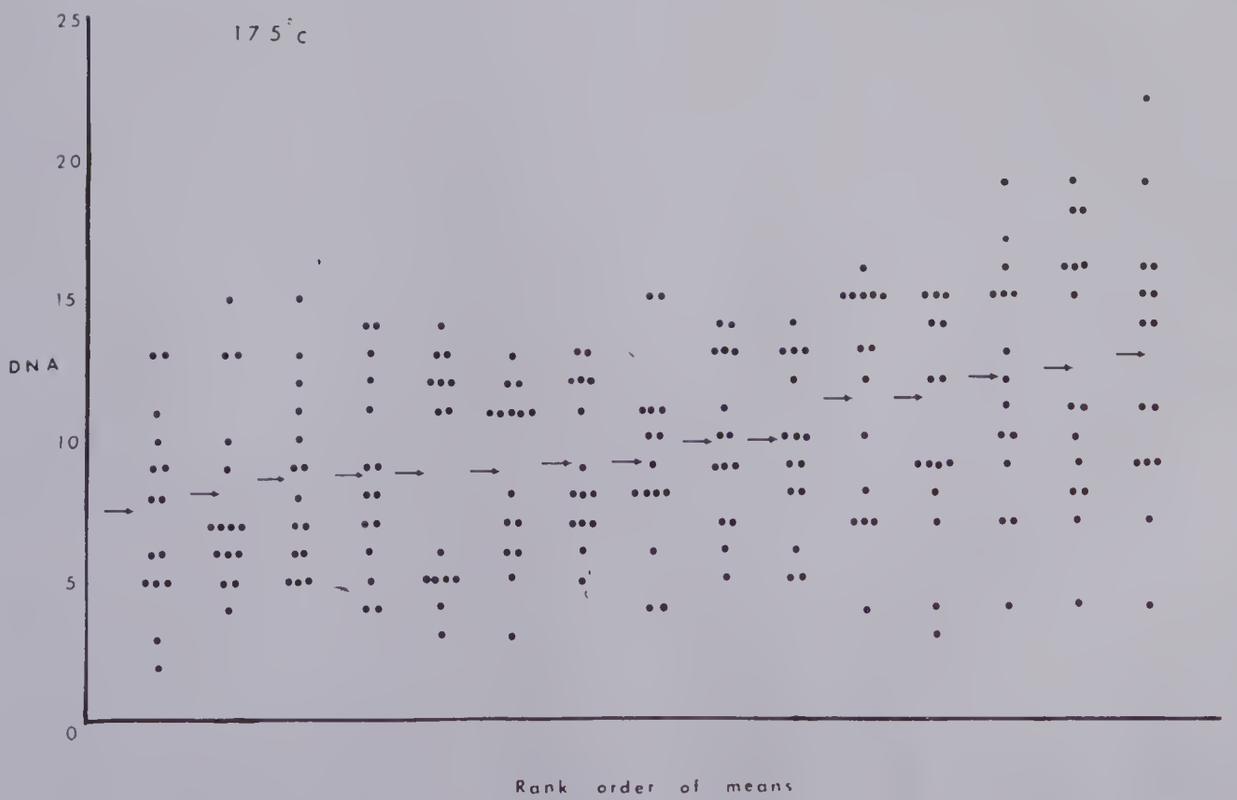
Source	Sum of Squares	df	Variance
Total	3250.72	224	--
Between slides	529.83	14	37.84
Residual	2720.89	210	12.95

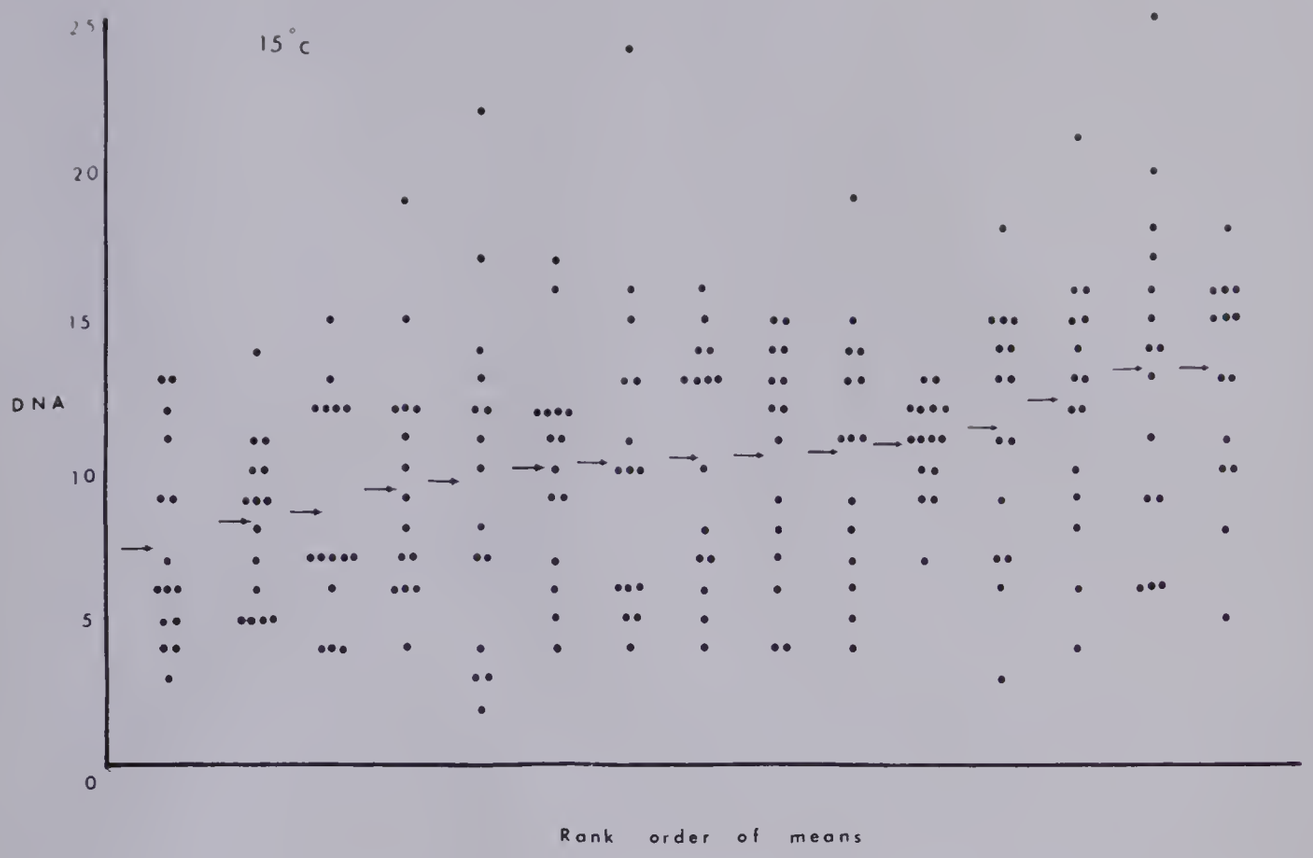
F = 2.92
$p < .01$

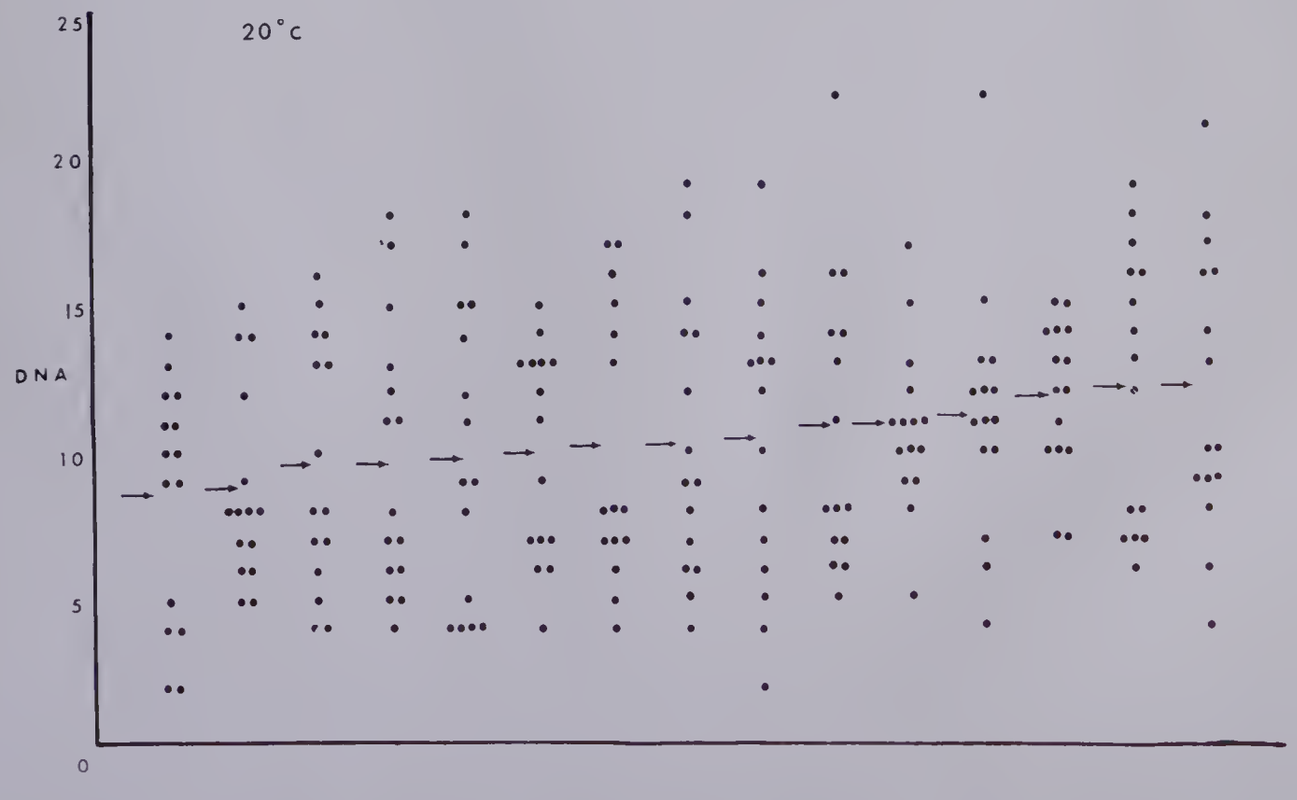
* Residual variance represents variance between nuclei within slides.

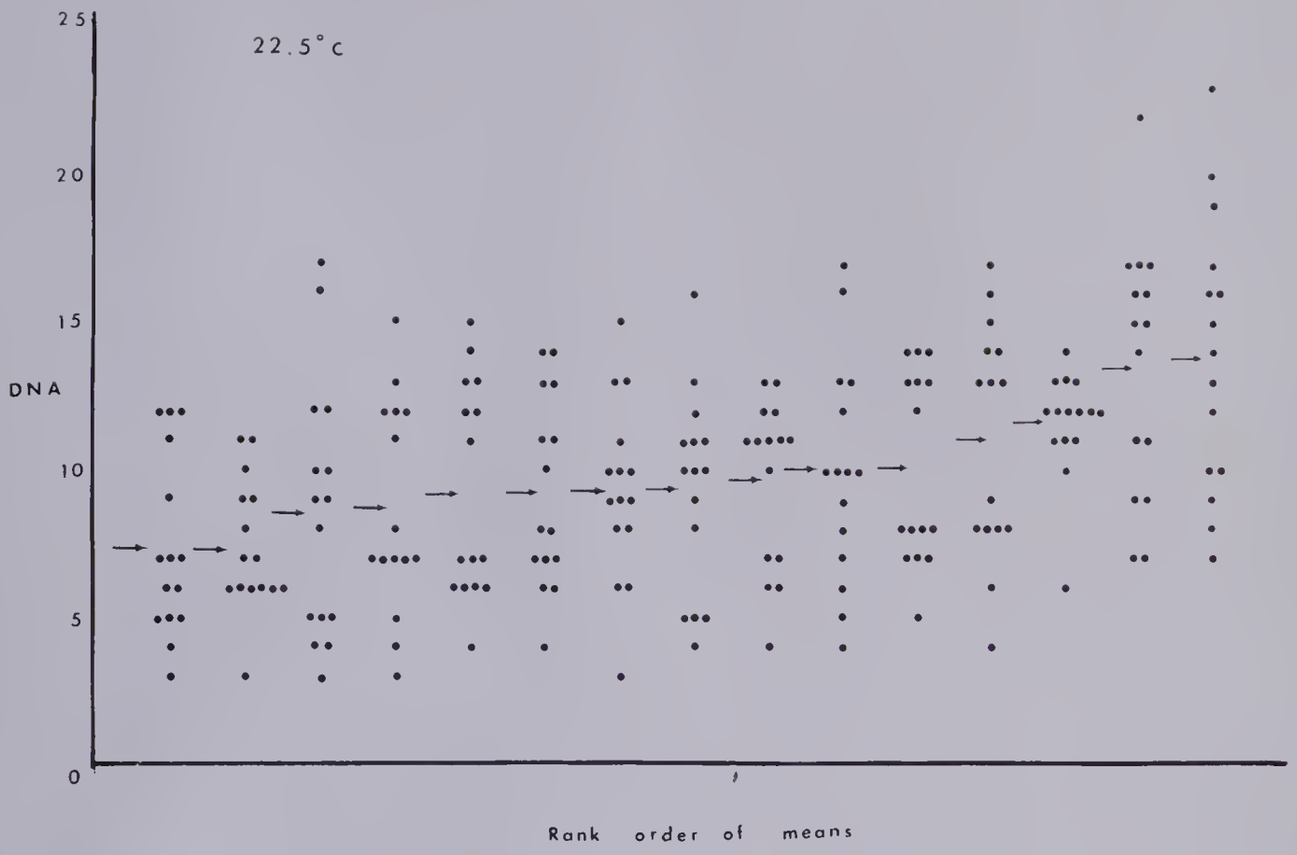
Figure 9. The computed DNA value for every nucleus measured at each of the temperatures. Each point represents one nucleus. The arrows denote the mean relative DNA content of the measured nuclei on each slide. The data from each slide has been grouped in one vertical array and these arrays ranked in increasing order of their mean values. The vertical axes represent the DNA content of the nuclei expressed in relative units.

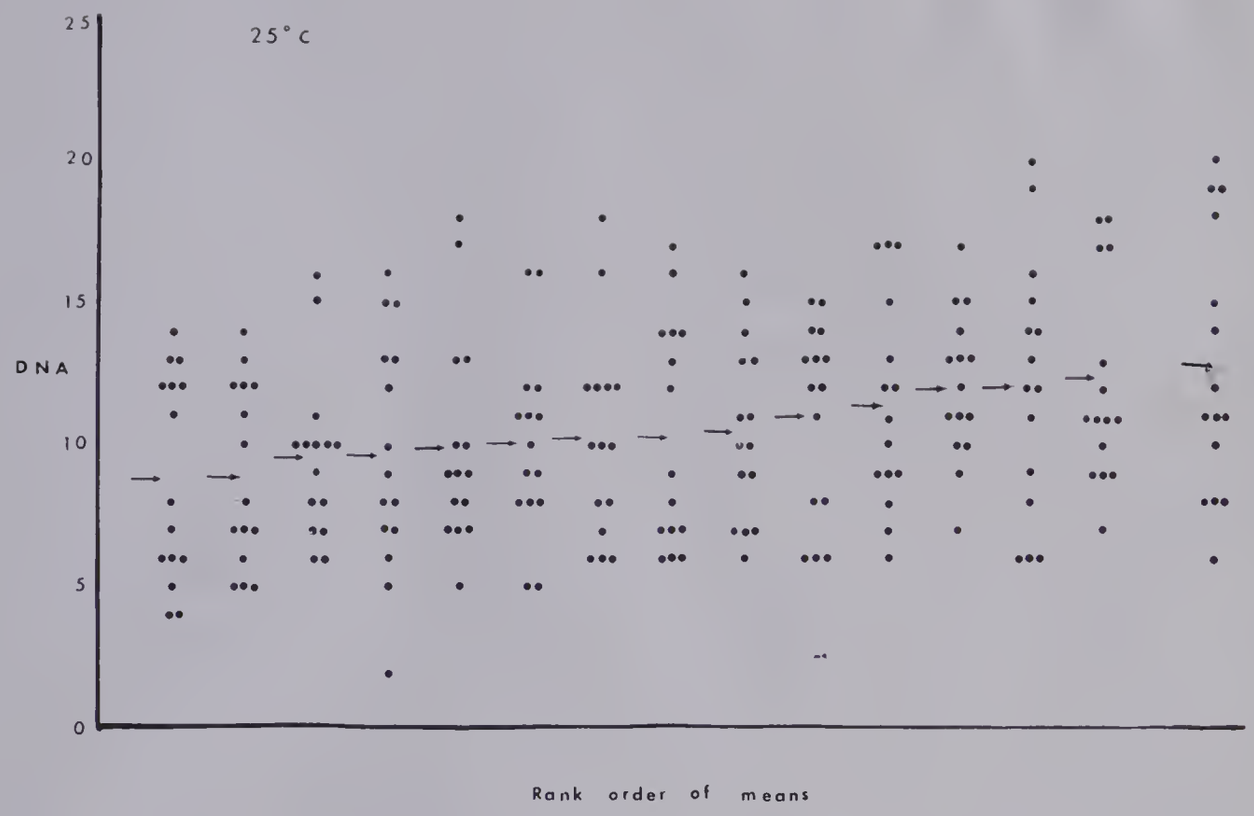

12 5°C
25
20
15
10
5
0
DNA
Rank order of means

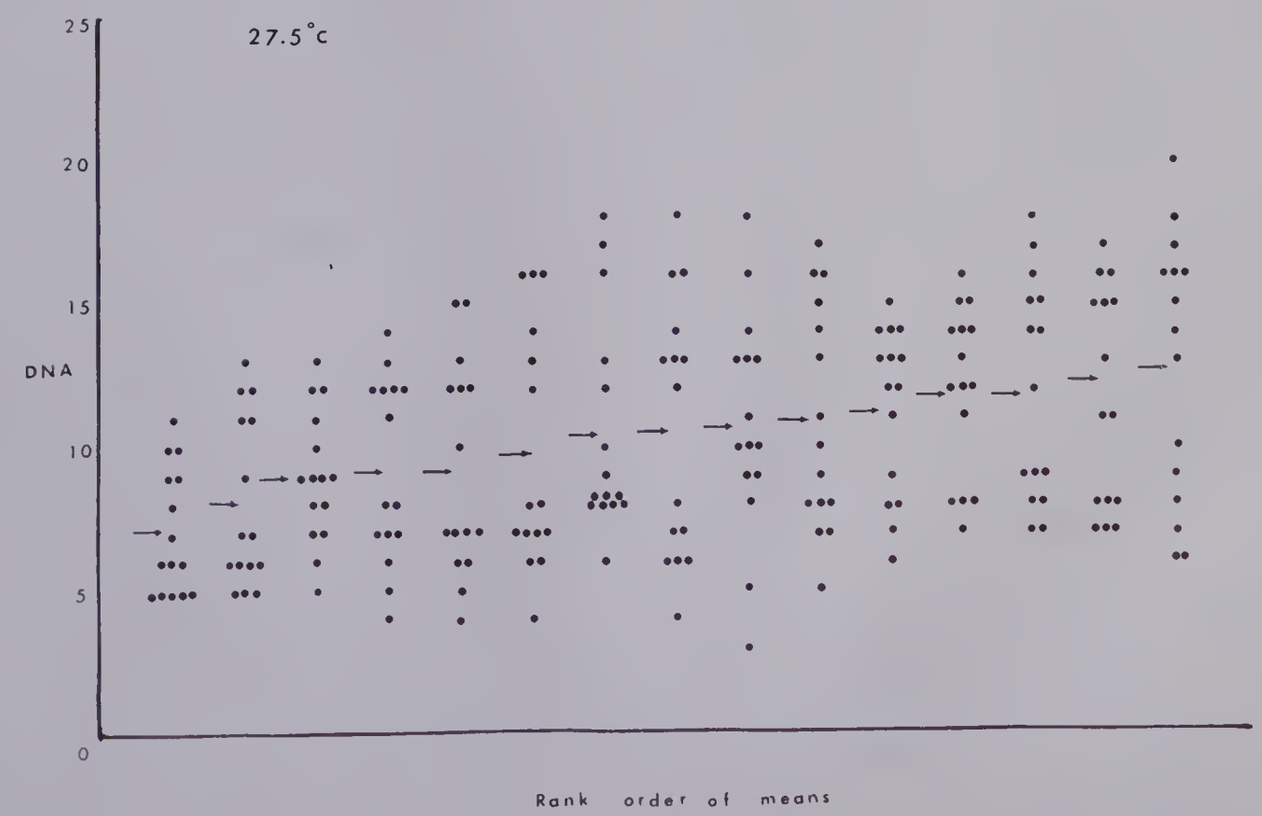

17 5°C
25
20
15
10
5
0
DNA
Rank order of means


15°c
25
20
15
10
5
0
DNA
Rank order of means


20°c
25
20
15
10
5
0
DNA
Rank order of means


22.5°C
25
20
15
10
5
0
DNA
Rank order of means


25°c
25
20
15
10
5
0
DNA
Rank order of means

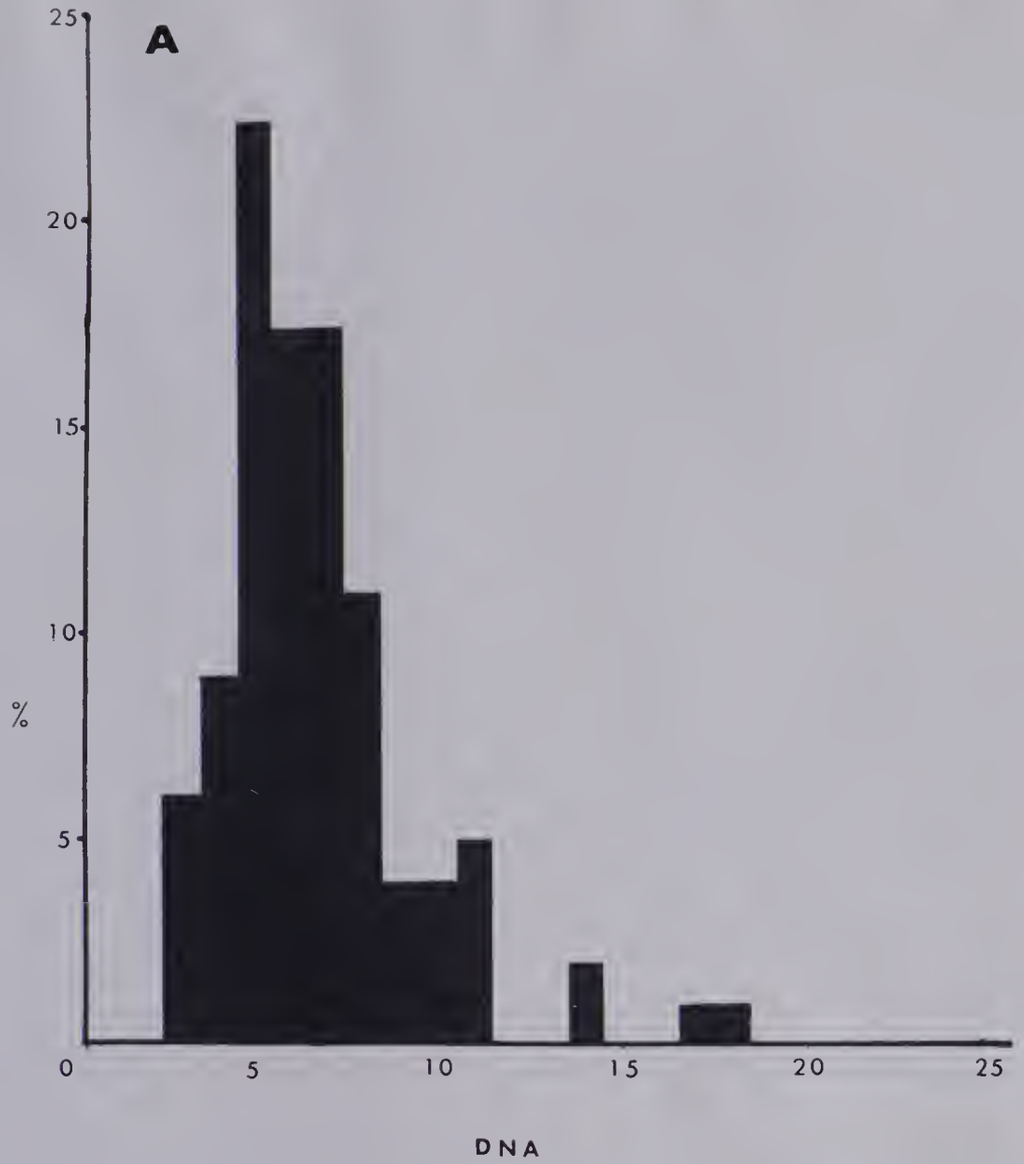

27.5°c
25
20
15
10
5
0
DNA
Rank order of means

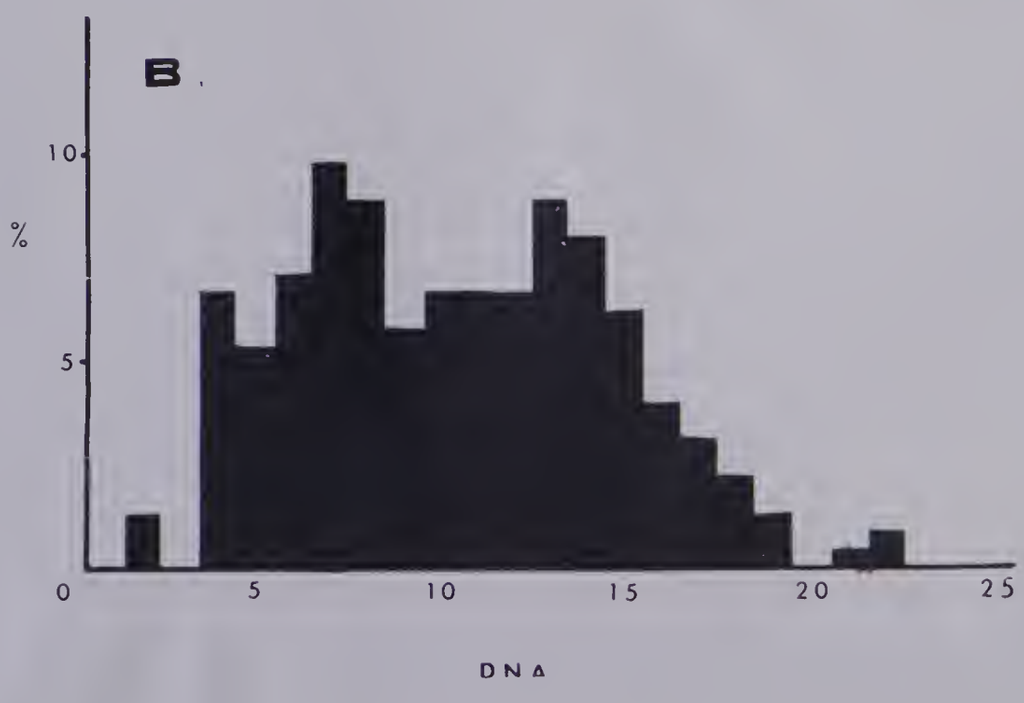
of individual measured nuclei on a slide are shown on the vertical axes. The horizontal axes portray a rank-order of slides in terms of the mean DNA value of the measured nuclei on each. In this figure it is possible to see the variation and distribution of individual nuclei on a slide and also make visual comparisons between slides. It can be seen that there is no obvious trend to the degree of variation found in relative DNA contents. However, it can be shown that the degree of variation between slides can be significant. The analysis of variance (Table II) shows that for five of the seven culture temperatures the degree of variation between slides is significant at the 1% level. The 12.5°C. sample shows extreme significance for the variation between slides. Figure 9 represents this visually as it can be seen that the distributions of values for relative DNA content for the slide of the highest mean and the lowest mean do not overlap.

The nuclei measured from each of the culture temperatures failed to segregate into discrete polytenic classes as can be seen in Figure 8. Although there is some evidence for peaks in these distributions (which would represent polytenic classes) in general these peaks are obscured by numbers of intermediate nuclei. These intermediate values could be due to experimental error, but, considering the numbers of nuclei still synthesizing DNA, it would be expected that true intermediates, representing nuclei in transition from one polyenic class to another, would be present. Table I shows that approximately 30% of the nuclei in all samples were synthesizing DNA as detected autoradiographically. The possible significance of the finding of many intermediates and the above mentioned autoradiographic result will be dealt with further in the discussion.

One test to show that the range of DNA values obtained for the prepupae is not artefact is the experiment described in the materials and methods on measuring feulgen-stained nuclei of mid third instar larvae. The results of this experiment are illustrated in Figure 10 (Page 39). It can be seen that the frequency of cells having lower relative DNA values is much greater in the mid third instar larval salivary glands than in the prepupal salivary glands. Furthermore, larval nuclei exhibiting larger values for DNA content are virtually absent. This result is fully expected if the photometric technique is sufficiently sensitive since it has been demonstrated that third instar larval nuclei show a lower mean DNA content than prepupal nuclei (Rodman, 1967a).

Figure 10. Distributions of relative DNA content in: A. a sample of 125 nuclei from mid third instar male larvae grown at 20°C. B. a sample of 225 nuclei from white prepupae grown at 20°C. See figure 8 for details.


A
25
20
15
10
5
%
0
5
10
15
20
25
DNA


B
10
5
%
0
5
10
15
20
25
DNA

DISCUSSION

Rodman (1967b) has hypothesized that initiation of new cycles of DNA synthesis in salivary glands of Drosophila melanogaster, leading to a higher level of polyteny, is a function of the larval state of the organism. She demonstrated that in a mutant stock, which resulted in an increased duration of larval life, a higher level of polyteny was achieved. It has been found in this study that culture temperature can affect duration of larval life; specifically, a lower culture temperature extends the duration of larval life. This finding coupled with the fact that the cell number in the salivary glands is established early in embryonic life leads to an a priori expectation with regard to the temperature effect on the control of DNA synthesis in the chromosomes of the salivary glands. If the initiation of cycles of DNA synthesis in larvae is merely a function of duration of the larval state then extending the larval period, by growing at lower temperatures, should give rise to these extra cycles.

An alternative possibility is that lowering the culture temperature and thereby extending the larval state will not affect the ultimate amount of DNA produced by a given developmental stage. This would be the expectation if the extension of larval life were due to a general slowing down of larval developmental rate which included a lowering of the rate of DNA synthesis. By the time of pupation at lower temperatures, then, the same amount of DNA would have been synthesized but at a much slower rate.

The results of this study would tend to favor the second possibility since no evidence is obtained which shows a significant rela-

tionship between culture temperature and DNA content of the nuclei. This finding partially seems to contradict the observation that larvae grown at low temperatures grow larger, have larger salivary glands and also larger salivary gland chromosomes. If it is not increased DNA content which accounts for the increase in size of these chromosomes other explanations must be explored. There is a possibility that these large chromosomes have an increased amount of protein or even that chromosomes grown at low temperatures are more susceptible to squashing and really only appear to be larger.

Rodman (1967c) also failed to show a dependence of DNA content per nucleus on culture temperature. The results of this study essentially confirm her findings but in a more convincing manner. She has shown that nuclei of glands from larvae reared at 17.5°C. and 25°C. can reach similar levels of polyteny. Her conclusions are based on four glands from each of her two culture temperatures and might be better taken to show that lowering culture temperatures does not invariably lead to differences in polytenic level. It has been demonstrated in this study (Figure 9) that even among the nuclei sampled from one temperature there is a great variation in mean DNA content from slide to slide. That this between slide variation can be significant has been shown in five out of the seven samples (Table II). From the results portrayed in Figure 9 it would be easy to compare glands with the highest mean DNA content from the 17.5°C. sample with glands of the lowest mean DNA content from the 25°C. sample to show that they varied significantly in their mean DNA content.

Examining Rodman's (1967c) data it can be shown that the mean DNA content per nucleus was notably higher in her 17.5°C. sample than in her

25°C. sample even though there are some nuclei at the highest level in each sample. This is due to a marked increase in the number of nuclei that reach the highest level of polyteny in her 17.5°C. sample. This study, using a greater range of temperature and a much larger sample, did not find any increase in average DNA content in the samples from the lower temperatures. In Rodman's study sampling error, through the use of a small sample, could account for the results. It must be stressed that Rodman probably realized this and concentrated on the ultimate levels of polyteny achieved, not on the average level attained.

The average DNA content of the nuclei of a sample would increase with an increased level of polyteny unless a greater portion of nuclei were also retained at a lower than normal level. In this study there is a slight tendency for more nuclei of the lower temperature samples to reach the highest measured level of polyteny found amoung all the samples. However, this does not result in a significantly higher mean DNA value for these samples since it appears that there are also more nuclei at the lower levels of polyteny than in most samples (see Figure 8 or 9). As seen in Figure 9, in the 12.5°C. sample, there is a definite tendency for a gland with an example of a nucleus with the lowest DNA content to exhibit a low mean value. A similar relationship is seen between 'high' nuclei and means of slides on which they appear. This results in an extremely significant between slide (gland) variance as shown in Table II. The between gland variance of the other temperatures is either nonsignificant or generally barely significant. In each of these cases sampling error, due to using only a 15 gland sample, could explain between gland variation. This would not explain the tremendous variance in the 12.5°C. sample. It is possible that part of

it might be due to experimental error but there is also the possibility that it is due to an organism-environmental interaction which has become evident at the lowest culture temperature.

As mentioned in the results section the calculated values for the various samples did not fall into discrete peaks representing presumed replications of a diploid value. However, the values where the maximum number of nuclei fall are roughly the same for every sample; in some cases represented by discrete peaks and sometimes rather obscured by intermediate values (see Figure 8). Even though the lack of discrete peaks could be due to experimental error there is good reason to believe that many of the intermediate values could be true intermediates in the process of synthesizing DNA, as suggested previously. One way of testing this would be an experiment in which cells are labelled with H^3-thymidine and then feulgen-stained. By measuring a sample of cells photometrically and then doing an autoradiographic analysis of the same cells that were measured photometrically it should be possible to ascertain if the frequency of label is higher over presumed intermediates or not. This would be the expectation if measured intermediate values were truly progressing from one polytenic level to the next highest.

However, from the work of Berendes and Keyl (1967) on polytene nuclei in brain ganglia of Drosophila hydei and from Rudkin's (1965) work on polytene nuclei of Drosophila melanogaster salivary glands, it is possible that discrete polytenic classes should not be expected in photometric measurements since both studies propose a differential replicative behavior for the heterochromatin and euchromatin. The findings of the above workers merely add an additional problem to

interpreting the numbers of intermediate values found in this study. The interpretation, in this case, will have to await the results of a definitive experiment, possibly the one suggested above in which a comparison could be made between measured intermediates and cells actively synthesizing DNA.

Autoradiographic analysis of a sample of glands from lower temperature cultures could have been expected to reflect extra synthesis of DNA resulting in a higher level of polyteny, if indeed it did occur. This depends on the degree of synchrony between the onset of puparium formation and the period of DNA synthesis (S period) in the salivary glands. If puparium formation is not synchronized with completion of a previous replication level, then progress towards an extra level of polyteny might still be underway as reflected by a higher percentage of cells undergoing DNA synthesis. On the other hand the 'S' period could be affected exactly in proportion to all other components of development by a lowering of growth temperature and therefore no unusual results would be expected by autoradiography. The results of this study favor the second possibility in that no trend is observed in the differences in the percentages of cells exhibiting label from among the different samples. This is in agreement with the results of the photometric study. However, the autoradiographic analysis did produce an unexpected result. The degree of variation found between glands of a sample in regard to the percentage of cells exhibiting label opens a new facet in the study of DNA synthesis in salivary glands of Drosophila.

Some attention has been given in trying to ascertain if this variation is due to experimental artefact or if it is a real phenomenon. Certain aspects of experimental error have been eliminated as described

in the results. At least one experiment (see Figure 6) suggests there is a biological basis for the variation. This experiment shows that there is a high correlation between the frequency of labelled cells in the two glands of a pair. In any case, temperature did not seem to affect the degree of variation since it is found to the same degree in the samples from all of the temperatures.

The results of this study seem to point out at least two components of biological variation of DNA synthesis in the salivary glands. One, as just mentioned, seems to be uninfluenced by the degree of environmental stress applied in these experiments. The other, manifest in the greater variance between slides in the 12.5°C. sample in regards to average DNA content, seems to be due to an organism-environment interaction.

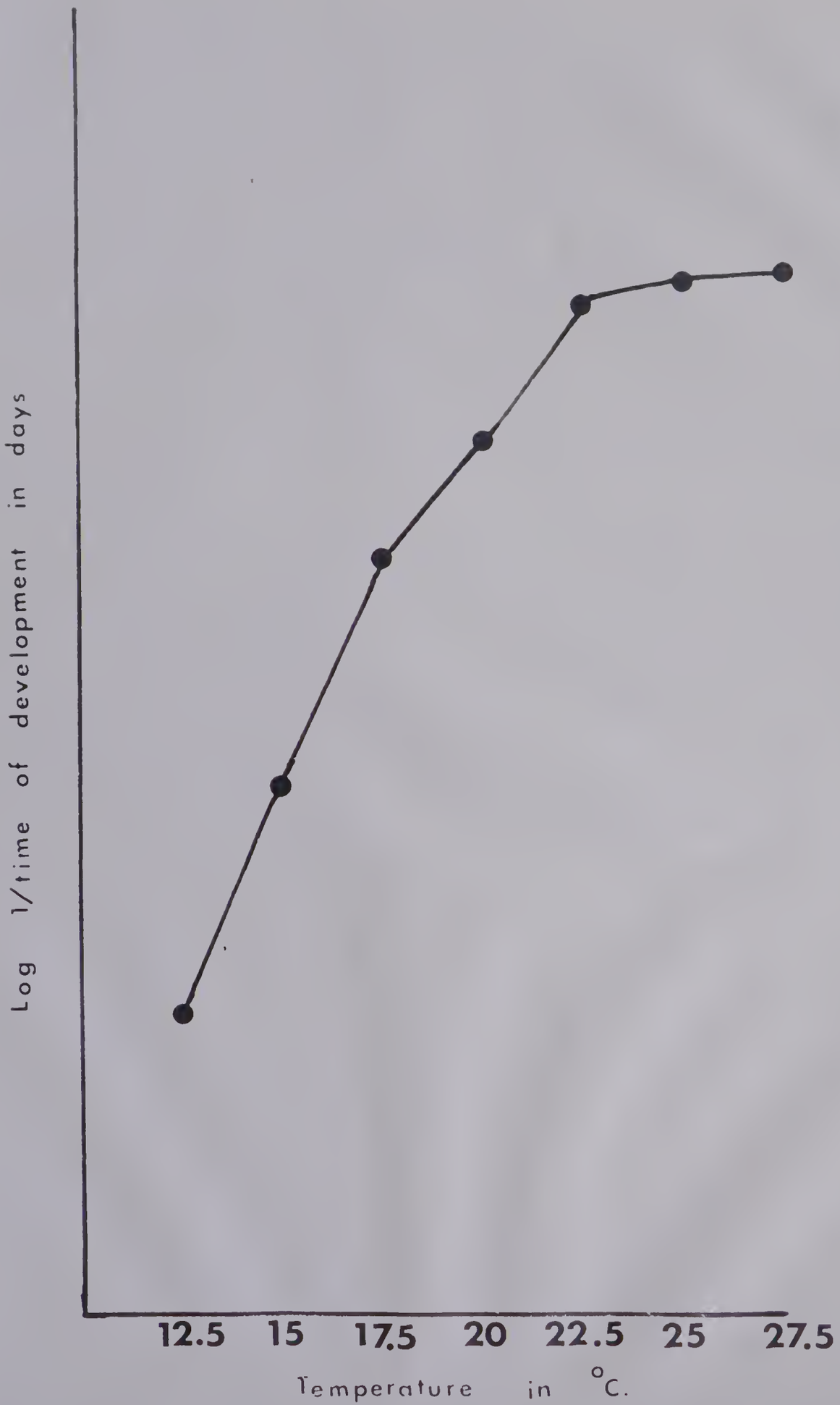
If the phenotype, in this case DNA content per cell, remains uniform in spite of genetic segregation or environmental manipulation it can be referred to by the general term canalization (Rendel, 1967). Since the role of DNA in the cell is so important it would not be surprising if the amount of DNA per cell is under definite control. Using Rendel's definition of phenotype, one can arbitrarily choose any measurable character and consider it as a phenotype. In this case the average amount of DNA per salivary gland nucleus grown at a standard temperature will represent a standard phenotype. Within limits, this phenotype is uninfluenced by 'make' (Rendel's term for all influences which can change phenotype). However, it can be shown that genetical manipulation can alter 'make' sufficiently to change this phenotype. Chen *et al* (1963) have demonstrated that the phenotype (DNA per nucleus) can be reduced. In the mutant 'lethal meander' (lme) of

Drosophila melanogaster it was shown that in 96 hour mutant larvae the DNA content per nucleus is about one-third that of wild-type. These larvae never pupate. Rodman (1967b) has demonstrated a case where the phenotype can be increased, for she has demonstrated an extra level of polyteny in the tumorous-head (tu-h) mutant of Drosophila melanogaster. This mutant exhibits a delay in pupation or frequently fails to pupate at all. From this it can be seen that significant deviation from the average DNA content per nucleus is associated with lethality, whether or not these mutations are in genes directly concerned with salivary gland metabolism.

Church and Robertson (1966a) have demonstrated that even in wild-type Drosophila there is a degree of polymorphism with respect to the amount of DNA per individual. Biochemical analysis of a number of lines has shown that selection under appropriate nutritional conditions can lead to considerable differences in the DNA content per individual. This could be said to result from segregation of polygenes affecting DNA synthesis. Thus it can be seen that segregation of polygenes can result in a significant change in 'make', either to increase it or decrease it.

Culture temperature could also influence 'make' (Rendel, 1967). The simplest expectation in regards to the influence of temperature in development rate, within the range where the organism is viable, is that each increment of temperature will result in a constant increase or decrease in development rate. It has been ascertained in this study that culture temperature can greatly alter time of development (Figure 4). Using Figure 4 to calculate development rate in regards to temperature change (Figure 11, Page 47) it can be seen that the simplest expectation does

Figure 11. Development rate of larvae as affected by culture temperature. This figure was compiled from the data of Figure 4.


Log 1/time of development in days
12.5
15
17.5
20
22.5
25
27.5
Temperature in °C.

not hold. It seems that change in temperature has less effect on development rate in the upper range of the temperatures used in these experiments. This flattening of the rate curve represents evidence for canalization. These temperatures probably correspond more closely to conditions in the wild for Drosophila. If temperature change can result in a partial escape from a canalization of the amount of DNA found in the average salivary gland nucleus, it is evident from Figure 11 that it is most likely to do so at temperatures farther from the canalized range; that is at the lowest culture temperatures used in these experiments.

On the basis of the above finding it is tempting to consider the greater variance of DNA contents of the 12.5°C. sample as being indicative of such a trend. Growing the larvae at this low temperature has had a disruptive influence on the organisms, allowing latent genetic differences to become manifest. That the variation of this sample is due to a genetic-environment interaction is fortified by two observations. Firstly, from Table II it can be seen that the between gland variation is extremely significant, which could represent genetic variation between individuals in regards to average DNA synthesized per cell. Secondly, it is only in the 12.5°C. sample that this very significant variation is found. If experimental error were the explanation for the variation, it is extremely difficult to rationalize why a similar error was not present in each of the other samples. Clearly, this problem will require more study of which a logical first step will be to attempt to grow prepupae at 10°C. and do photometric measurements. However, from the results of this study it appears that a case can be made for genetic polymorphism in wild-type individuals with respect to

how much DNA the average salivary gland cell will contain.

With respect to the heterogeneity of the autoradiographic data, there is no basis for considering an environmental influence. There is, however, one possible biological explanation for the variation in autoradiographic behaviour which commands some attention. It is known that there is a rapid drop in labelling from third instar larvae to prepupae (Rodman, 1968). A slight difference in phasing between individuals in the time of cessation of initiation of DNA synthesis relative to formation of white prepupae would result in the variation found in labelling frequencies of the glands from different individuals and since the frequency of labelling drops off rapidly in this interval a large variation would result from a slight difference in phasing. This variation may be genetic in nature and it is also possible that it is related to the variation found in DNA contents of the samples. However, one can not be definite about this since the variations could also have resulted from other biological factors, not determined by genetics.

In summary, the following conclusions can be made. Firstly, changing the culture temperatures within the limits of this experiment, which approach the limits of viability for Drosophila, has no significant effect on the ultimate highest level of polyteny attained; nor does culture temperature seem to alter the mean DNA levels that salivary gland cells will attain. Secondly, the fraction of cells synthesizing DNA is uninfluenced by culture temperature. Thirdly, the finding of a great variation in labelling frequency among individual salivary glands makes it unsafe to assume biological homogeneity of a sample of salivary glands. Lastly, this study suggests the hypothesis that there is genetic

heterogeneity in wild-type Drosophila melanogaster with respect to the amount of DNA which can be synthesized in the salivary glands.

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